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Review

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Photolabile Linkers for Solid-Phase Synthesis

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ABSTRACT. Photolabile linkers are the subjects of intense research, because they allow the release of the target molecule simply by irradiation. Photochemical release of synthesis products is often facilitated without additional reagents under mild reaction conditions, which may even be environmentally friendly and appealing in the context of greener chemistry. The mild conditions also allow for applications of released material in subsequent biological screening experiments, where contamination with cleavage reagents would be detrimental. This review pays attention to the increasing number of photolabile linkers developed for solid-phase synthesis and release, and covers: (i) *o*-nitrobenzyloxy linkers, (ii) *o*-nitrobenzylamino linkers,

(iii) α -substituted *o*-nitrobenzyl linkers, (iv) *o*-nitroveratryl linkers, (v) phenacyl linkers, (vi) *p*-alkoxyphenacyl linkers, (vii) benzoin linkers, (viii) pivaloyl linkers, (ix) other photolabile linkers.

INTRODUCTION

Originally pioneered by Merrifield for the synthesis of peptides,[1] solid-phase organic synthesis (SPOS) is a well-established technique that offers unique advantages over conventional solution-phase methods. With experimental simplicity, reactions are driven to completion with excessive amounts of reagent, and even highly polar and polymeric organic compounds are readily handled through multiple reaction steps, and easily isolated following release of product from the solid support. Since the introduction of solid-phase methods half a century ago, many laboratories have focused on the development of technologies and chemistry suitable for solid-phase synthesis, which has resulted in a remarkable outburst of chemical transformations that can be applied for the routine synthesis of organic molecules on solid support.[2, 3, 4, 5, 6] This has led to the application of solid-phase synthesis for the generation of molecular libraries in academia and industry to identify new bio-active molecules, drugs, catalysts and other fine chemicals.[7, 8] Solid-phase synthesis is the cornerstone of combinatorial chemistry, either through the generation of nucleic acid libraries for biological display, or in in-bead based split-pool approaches.

The use of solid supports in organic synthesis relies on several interconnected requirements. The substrate containing multiple chemical handles must be attached to the solid support, and then modified through multiple chemical transformations before controlled release

of the target product. Conventional linkers normally used in SPOS typically rely on harsh cleavage conditions, comprising strong acids, bases or nucleophiles, which can pose compatibility problems with more sensitive molecular structures. Inherently, the range of chemical transformations available for the synthesis of any given compound is restricted by linker. Photolysis offers a method of linker cleavage, which is fully orthogonal to conventional chemical methods and therefore, holds a unique promise for applying an expanded range of chemical transformations to build structurally diverse compounds on solid support. Photolabile linkers generally do not need acidic, basic or metal-assisted activation for cleavage, and photochemical substrate release is often facilitated without additional reagents under mild reaction conditions, that may even be environmentally friendly and appealing in the context of greener chemistry. The mild conditions may, furthermore, become attractive for applications of released crude material in subsequent biological screening experiments, where contamination with cleavage reagents would be detrimental. Recent advances in LED lighting technology have significantly increased the ease with which photochemical transformations may be achieved, and the field has witnessed a markedly increased interest in recent years. Herein, we provide an extensive overview of the synthesis and applications of photolabile linkers for solid-phase synthesis.

PHOTOLABILE LINKERS

o-Nitrobenzyloxy linkers

Nitrobenzyl-based linkers are often considered the most commonly used linkers. Their cleavage mechanism is traditionally identified as a Norrish type II reaction, as the photochemistry was described by Norrish in 1935.[9] Norrish demonstrated that an incident

photon (300-350 nm) breaks the N=O π -bond in the nitro-group, forming a diradical excited state. During the photoisomerization, the activated oxygen of the nitro group abstracts a proton from the benzylic position, forming the *aci*-nitro compound, which subsequently rearranges to afford an *o*-nitrosobenzaldehyde, thereby releasing the product (Figure 1).

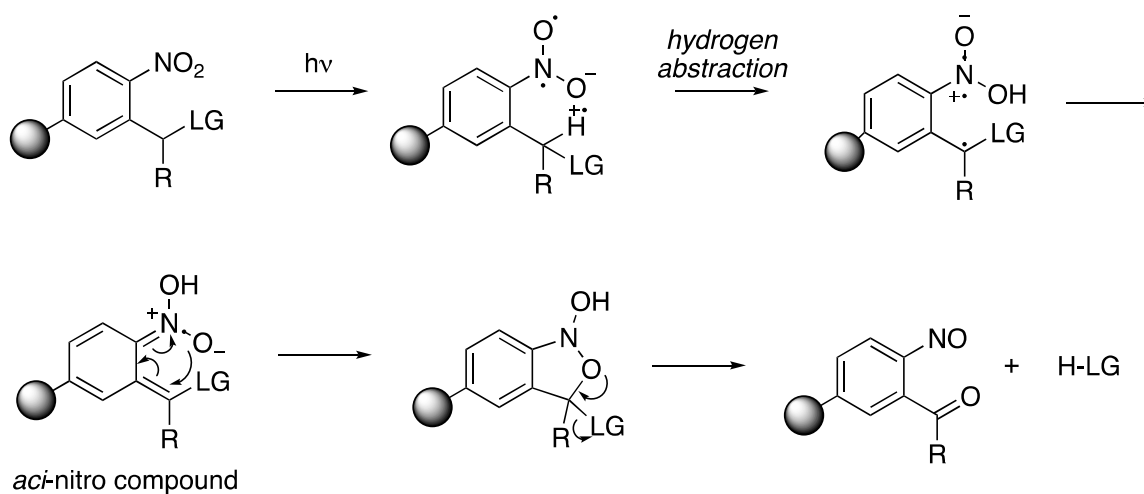
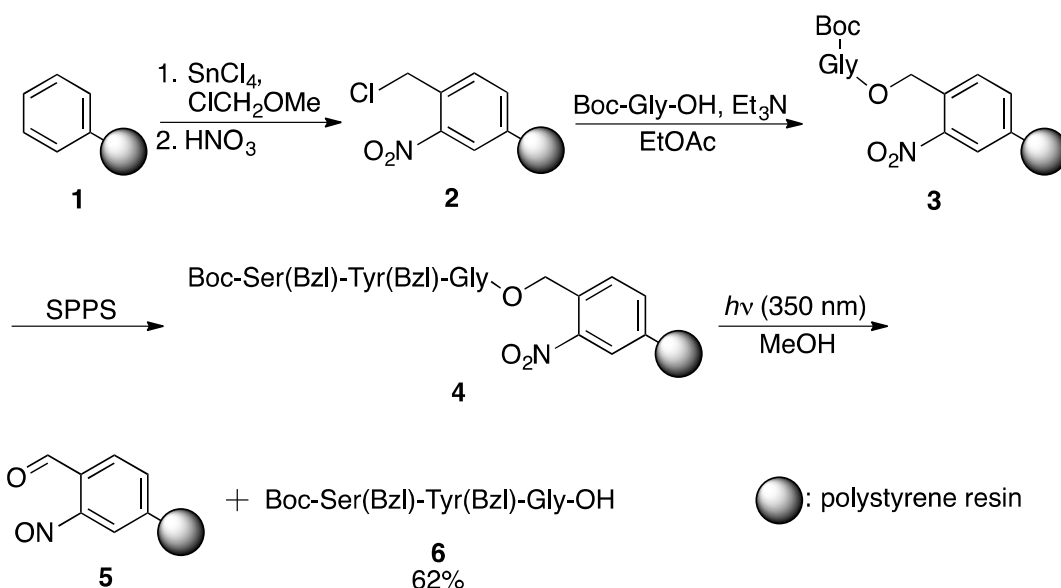


Figure 1. Norrish Type II mechanism for the photo cleavage of a 2-nitrobenzyl-based linker.

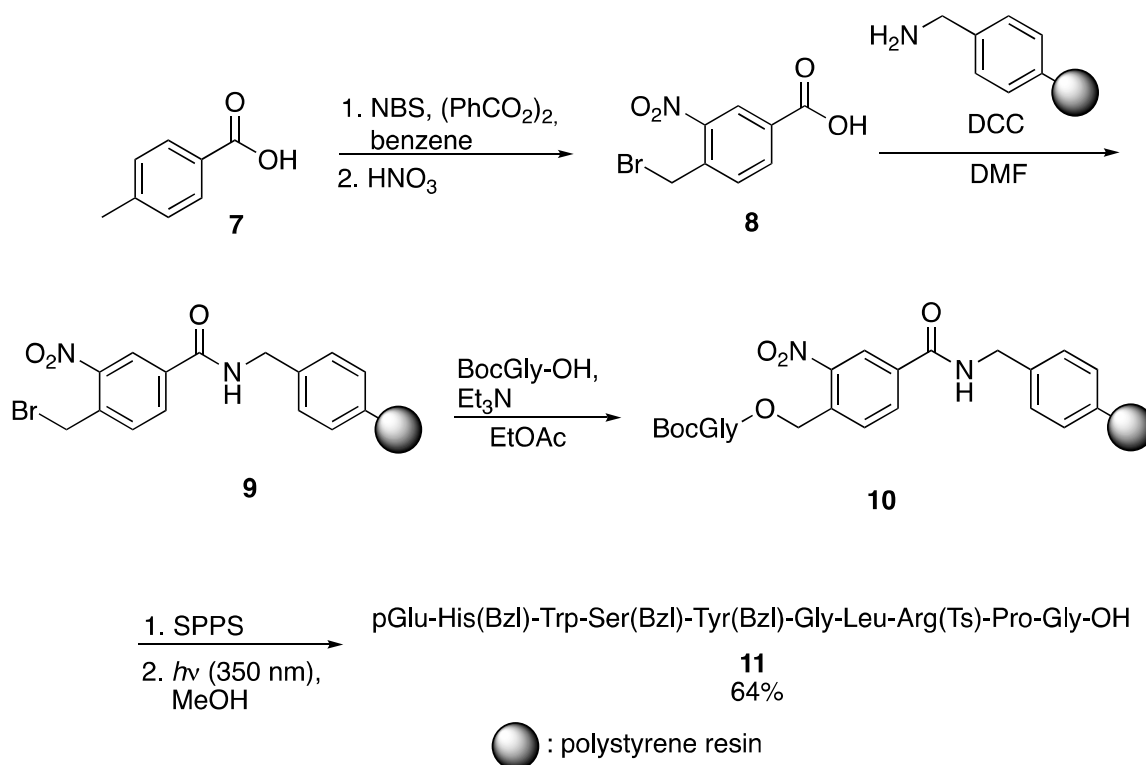
Overall, nitrobenzyl-based linkers are highly general, and the list of functional groups that can be released are now extensive. The scope includes, but is not limited to phosphates, carboxylates, carbonates, carbamates, and alkoxides. Research efforts towards raising the chemical and quantum yields of nitrobenzyl-based linkers have led to the identification of several beneficial modifications, which will be discussed in the following sections.

The first reported example of a photolabile linker was the *o*-nitrobenzyloxy based resin pioneered by Rich and Gurwara in 1973.[10] The resin was constructed using a Friedel-Crafts-type chloromethylation of the aromatic rings of polymer **1**, cf. Scheme 1. A nitration, as described by Merrifield,[1] made the resin ready for attachment of the first amino acid at the C-terminus affording **3**. After standard SPPS, photolysis released the first peptide constructed on a photolabile linker in a yield of 62%.



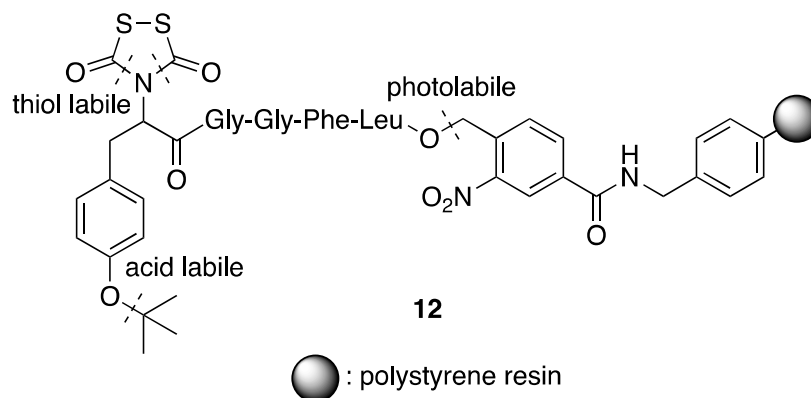
Scheme 1. First solid-phase synthesis and application of a photolabile linker based on the *o*-nitrobenzyloxy functional group.

However, it became clear that this strategy was not applicable to the synthesis of peptides incorporating more than four amino acids.[11] Over-nitration of the resin apparently caused enhanced polarity of the resin resulting in reduced swelling in organic solvents. In an attempt to find a solution to this limitation, a new strategy was developed where the linker **8** was prepared separately and then coupled to the resin,[11] cf. Scheme 2. Construct **9** was then set up for attachment of the first amino acid and following standard SPPS, the decapeptide **11** was released in 64% yield.



Scheme 2. Synthesis and application of an *o*-nitrobenzyloxy linker.

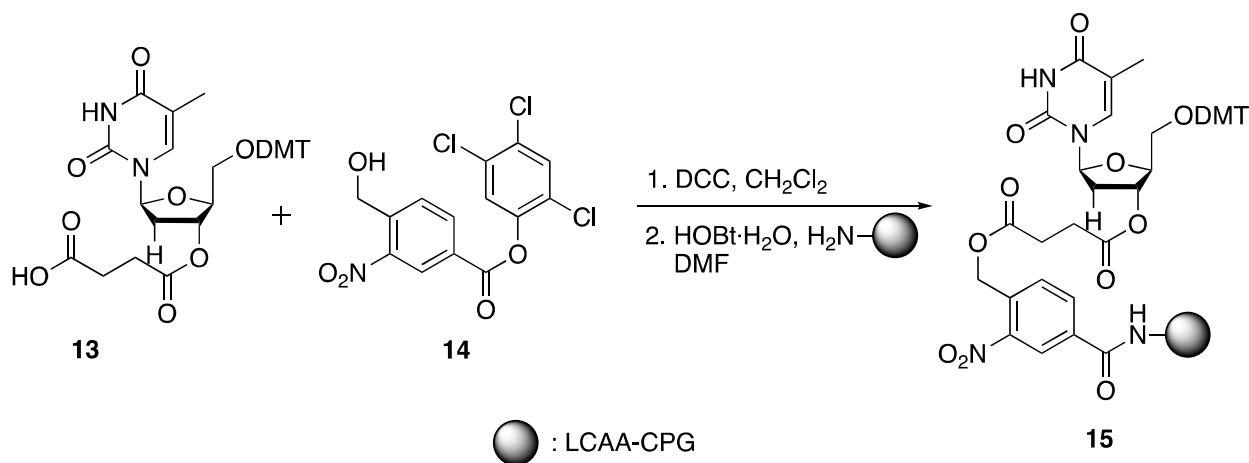
Later, Merrifield and coworkers have used linker **8** for the preparation of multi-detachable resins [12, 13]. Barany and Albericio have applied the linker in an orthogonal protection group scheme for solid-phase organic synthesis [14], where the photolabile construct **12** contained a phenolic *t*-butyl group and a dithiasuccinoyl *N*- α amino protecting group, both of which are photochemically inert and mutually orthogonal (Scheme 3).



Scheme 3. Three-dimensional and orthogonal protection of a resin-bound peptide.

Different variations of this linker construct have been explored, e.g. with a phenyl group in the benzylic position,[15, 16] or a glycine unit as an internal standard.[17] Pillai *et al.* has also reported use of the *o*-nitrobenzyloxy linker with a PEG spacer in between the photolabile cleavage site and the solid support.[18] In a study aiming for the synthesis of encoded small molecule libraries, Ohlmeyer and coworkers used the *o*-nitrobenzyloxy linker to facilitate easy release of the synthesized compounds under conditions optimal for direct use in biological assays.[19]

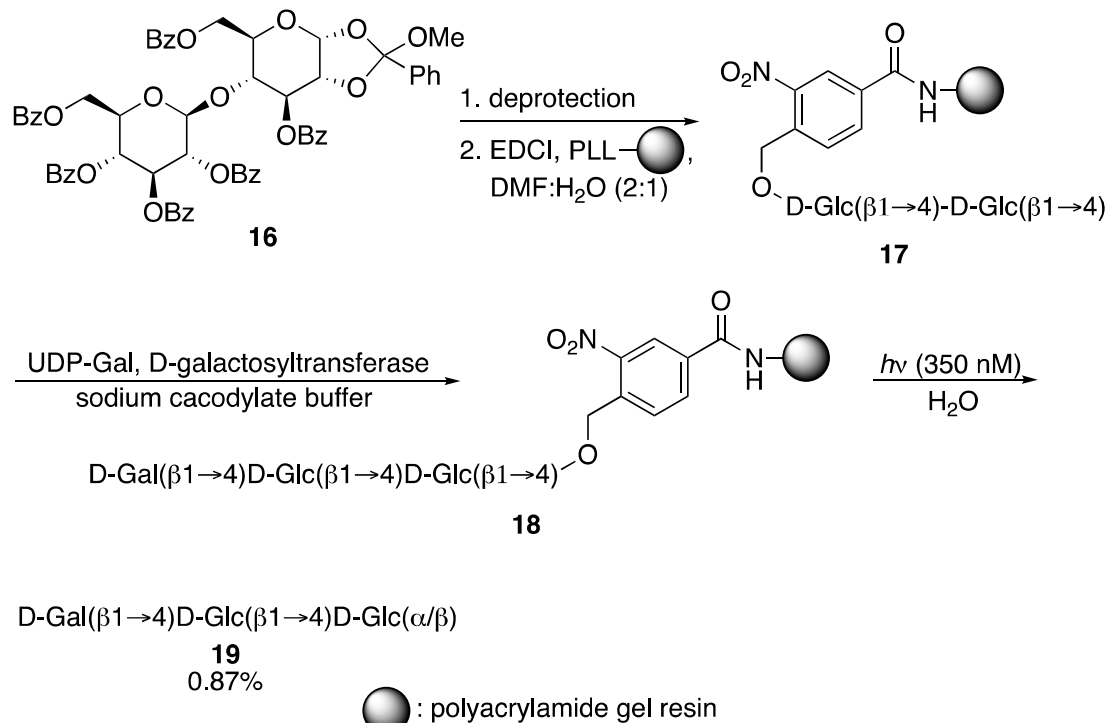
In 1993 Greenberg and coworkers demonstrated the application of *o*-nitrobenzyloxy-based linkers for oligonucleotide synthesis.[20, 21, 22] A DCC-mediated coupling of succinatothymidine **13** to the trichlorophenyl ester **14**, followed by coupling to a long-chain alkyl amine controlled pore glass, LCAA-CPG, support in the presence of HOBt led to construct **15**, which allowed for the synthesis of oligonucleotides (Scheme 4).



Scheme 4. Solid-phase oligonucleotide synthesis using a photolabile linker.

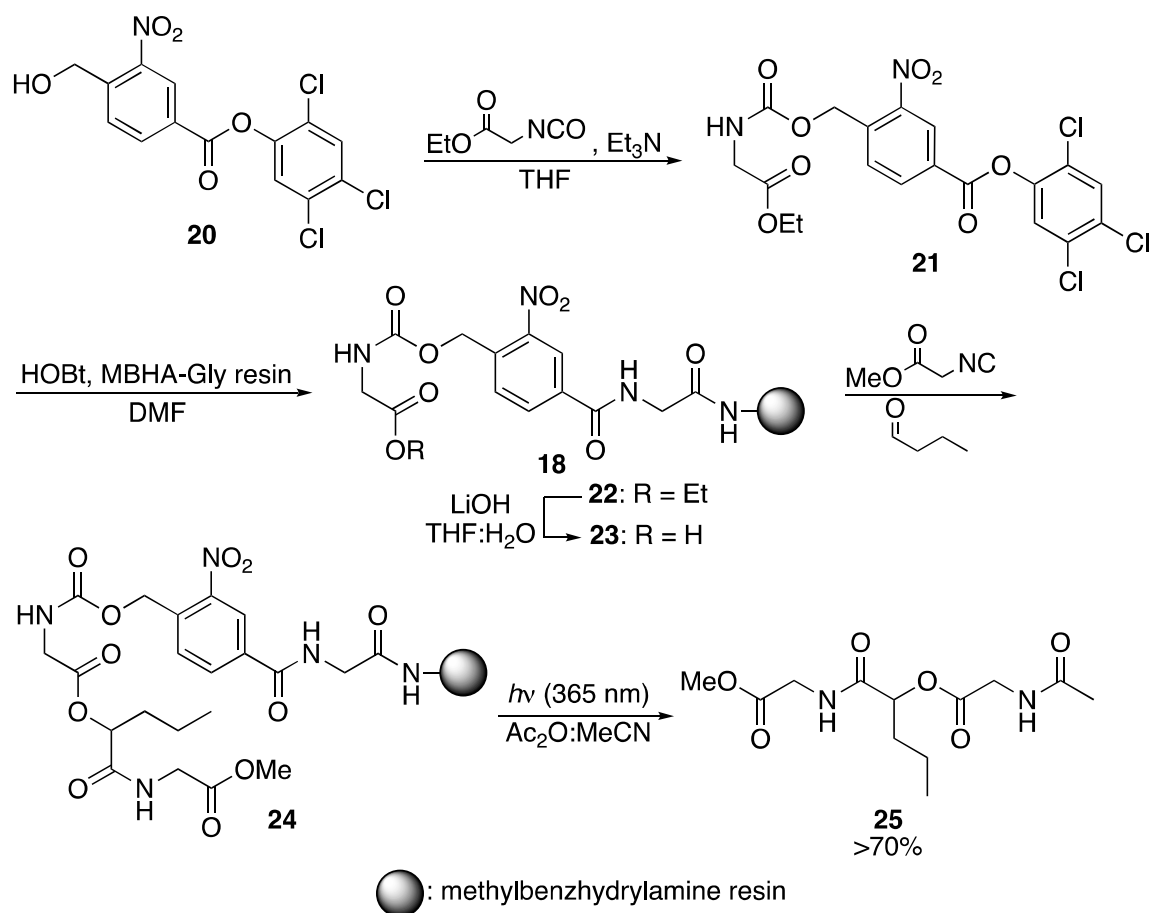
Alcohol-containing groups can also be attached to a *o*-nitrobenzyloxy linker as either carbonates,[23] or ethers,[24] for example in oligosaccharide synthesis. In pioneering work by Zehavi *et al.*,[25] however, a rather low isolated yield of oligosaccharides was obtained (e.g. 0.87 % of **16**, Scheme 5). The low yield was ascribed to low accessibility of amine handles in the

aminoethyl substituted polyacrylamide gel resin used. Later, Nicolaou *et al.*, [26, 27] Kantchev and Parquette [28] demonstrated the use of the *o*-nitrobenzyloxy linker in oligosaccharide synthesis, allowing for a higher isolated yield of the desired oligosaccharides.



Scheme 5. Oligosaccharide synthesis utilizing a photolabile *o*-nitrobenzyloxy linker.

Using the linker construct **14**, amines can be attached through a carbamate moiety as shown by Armstrong *et al.* for combinatorial library synthesis using the Passerini reaction (Scheme 6). [29]

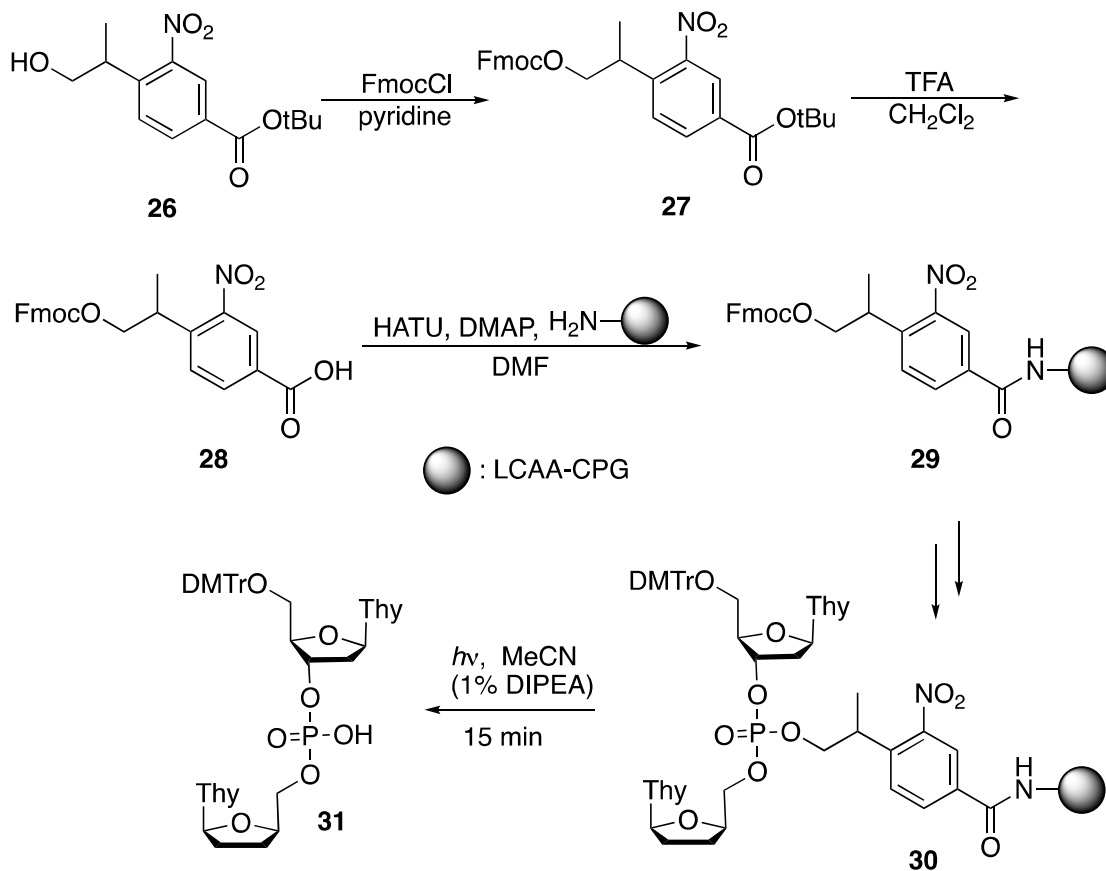


Scheme 6. Photolabile *o*-nitrobenzyloxy linker attached to an amine through a carbamate moiety.

2-(2-Nitrophenyl)propyloxycarbonyl (NPPOC) linker

To lower the photolysis time required for efficient cleavage compared to *o*-nitrobenzyl linkers, Damha *et al.* hypothesized that extending the chain containing the reactive hydroxyl handle by one carbon atom and branching it to generate a tertiary benzylic carbon center would be beneficial.[30] The effects of a substituent in the benzylic position are consistent with the relative rates of hydrogen abstraction from alkyl carbon by a photo-activated *o*-nitro group: primary < secondary < tertiary.

To investigate this feature, Damha et al. prepared linker **28**.^[30] Starting from **26**, linker **28** was synthesized in two steps followed by attachment to the solid support (Scheme 7). With construct **29** in hand, Damha synthesized a library of oligoribonucleotides and found that the target molecules indeed were photolytically released from the linker in only 15 minutes at room temperature.



Scheme 7. Photolabile *o*-nitrobenzyloxy linker for incorporation of a tertiary benzylic carbon.

Despite the structural similarity between *o*-nitrobenzyl linkers and **28**, the release mechanism differs markedly. The generated *aci*-nitro intermediate rearranges to *o*-nitrostyrene with β -elimination of the linker group,^[31] as illustrated in Figure 2.

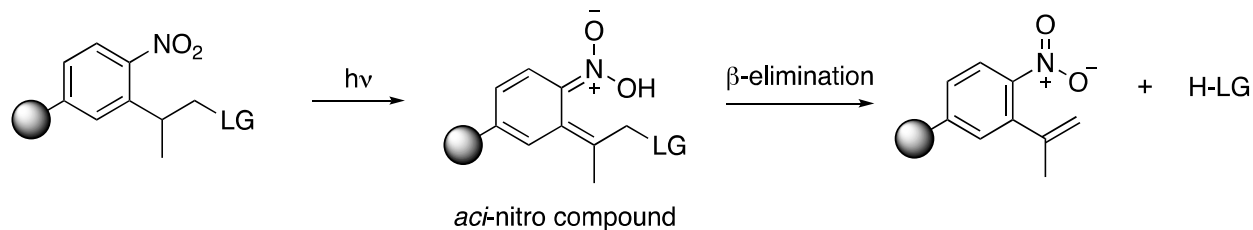
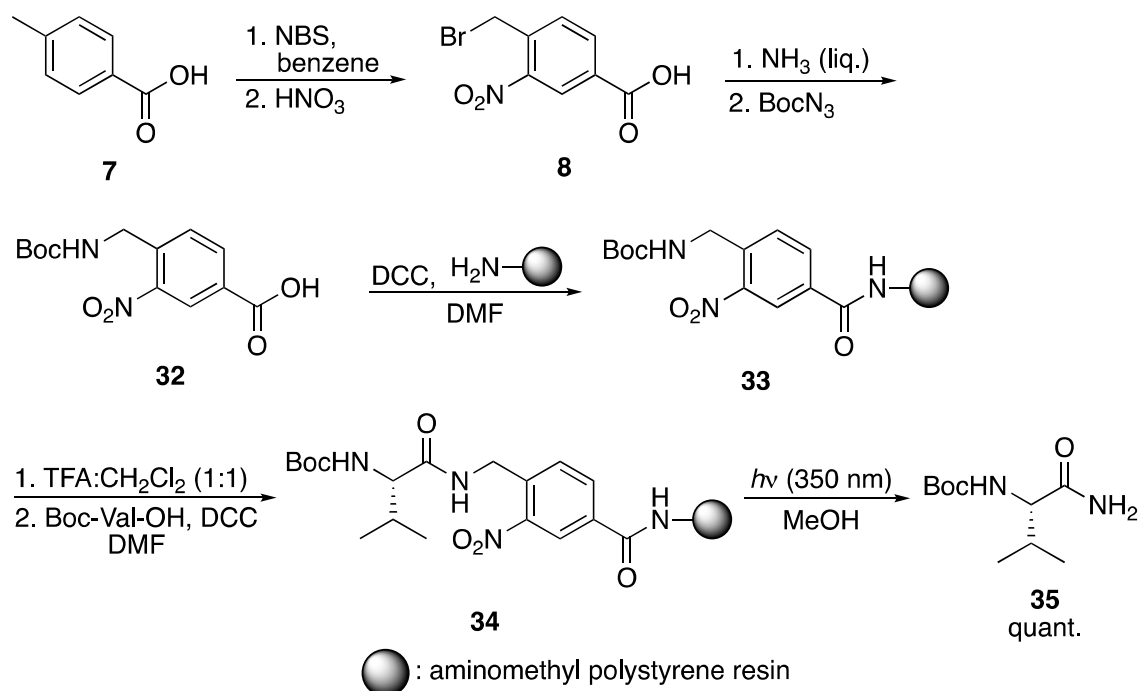


Figure 2. Mechanism for the photocleavage of NPPOC derivatives.

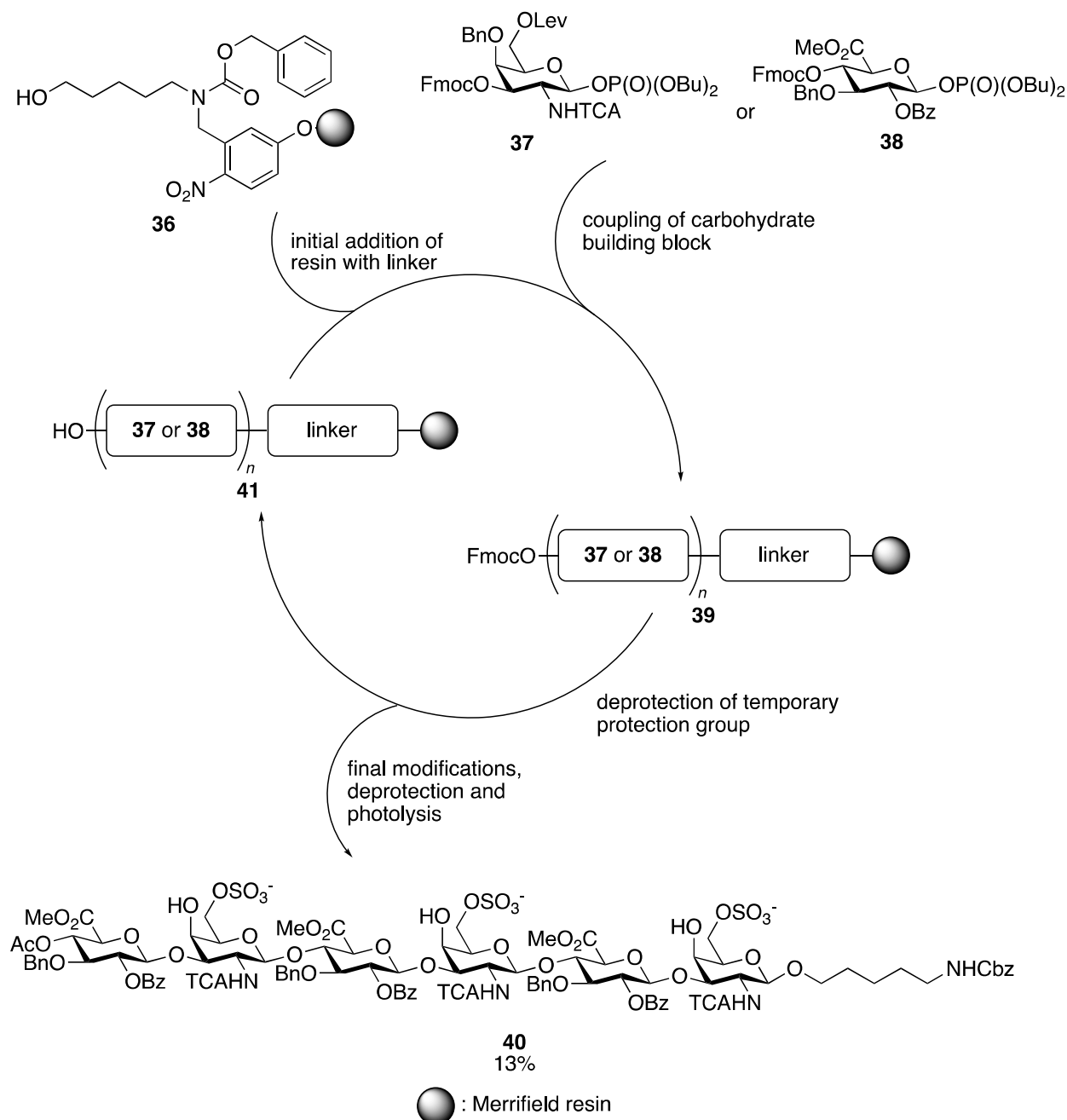
o-Nitrobenzylamino linkers

Taking into account that many biologically active peptides possess a *c*-terminal primary amide, Rich and Gurwara developed a variation of the *o*-nitrobenzyl-based linker that upon photolysis releases amides (Scheme 8).[32]. Starting from *p*-toluic acid, bromination followed by nitration led to **8**. The bromide **8** was then aminated and immediately Boc-protected using Boc-azide to furnish **32**. After attachment to the resin and deprotection, Boc-Val-OH was attached. After photolysis Boc-Val-NH₂ (**35**) was provided in quantitative yield. Furthermore, it was shown that a decapeptide could be prepared and released in high purity and good yield (65%). Another synthetic route to provide **32** from 4-aminomethylbenzoic acid was presented by Barany and coworkers.[33] Ajayaghosh and Pillai demonstrated the possibility of releasing secondary amides by substituting the linker precursor **8** (or the corresponding chloride) with a primary amine instead of ammonia.[34, 35, 36] Hinterstein, *et al.* used an *o*-nitrobenzylamino linker in their on-bead screening of a one-bead one-compound library in the search for nuclear import inhibitors.[37]



Scheme 8. Photolabile *o*-nitrobenzylamino linker for release of amides.

Recently, Seeberger and coworkers developed an approach for the automated synthesis of glucosaminoglycans utilizing an *o*-nitrobenzylamino linker.[38] Iterative cycles of coupling carbohydrate building blocks **37** and **38** to the linker construct **36**, yielded, in 16 steps, the hexasaccharide **40** in 13% yield after photocleavage in a continuous flow photoreactor, cf. scheme 9.

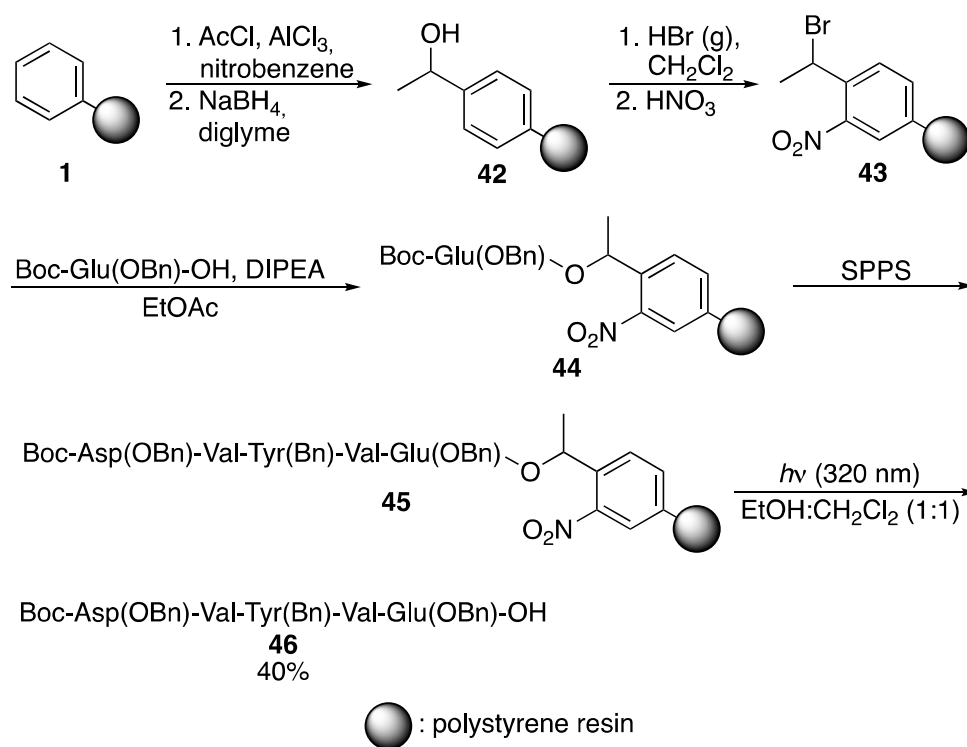


Scheme 9. Automated solid-phase synthesis of glucoseaminoglycans using an *o*-nitrobenzylamino linker.

Later, the same linker construct (**36**) was used for the synthesis of a 30-mer mannoside[39] and for the synthesis of β -(1,3)-glucans.[40]

α -Substituted *o*-nitrobenzyl linkers

The *o*-nitrobenzyl-based linkers presented so far suffer from disadvantageous formation of side products, such as *o*-nitrosobenzaldehyde, which is a very reactive species prone to polymerization, forming a highly colored product, which may act as an undesired internal light filter that affects the efficiency of the photolytic release.[41] To solve this issue, Ajayaghosh and Pillai prepared resin **43**, featuring a secondary benzyl alcohol as reactive handle for peptide attachment (Scheme 10).[41]

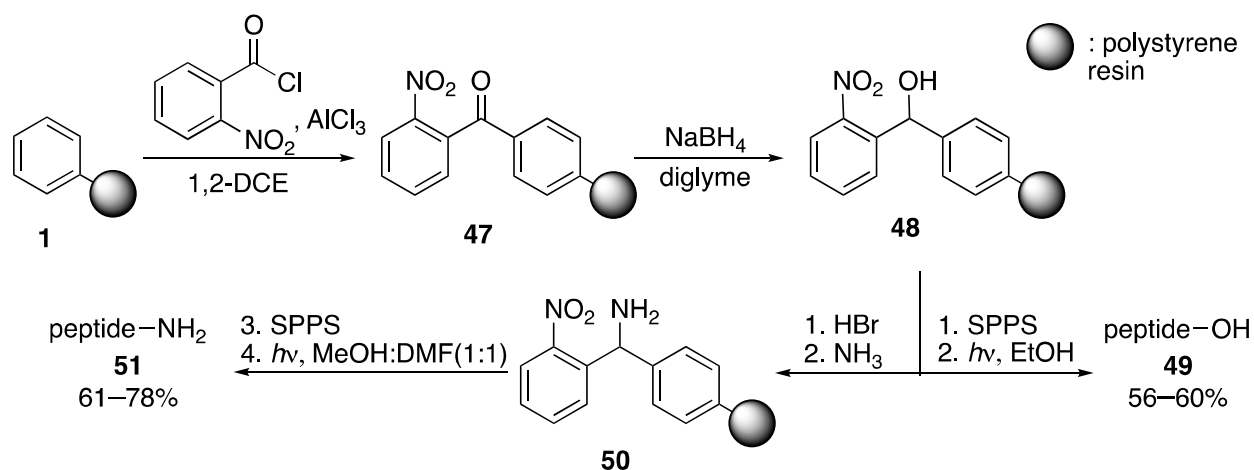


Scheme 10. Integral α -substituted nitrobenzyl-derived photolabile linker used for the synthesis of pentapeptide **46**.

Resin **1** was subjected to Friedel-Crafts-type acylation, followed by reduction to the alcohol-functionalized resin **42**, which after substitution and nitration was transformed into the bromo-functionalized integral linker **43**. This linker was used for the synthesis of pentapeptide **46** in a yield of 40%. This resin, however, suffered from problems similar to those observed with Rich

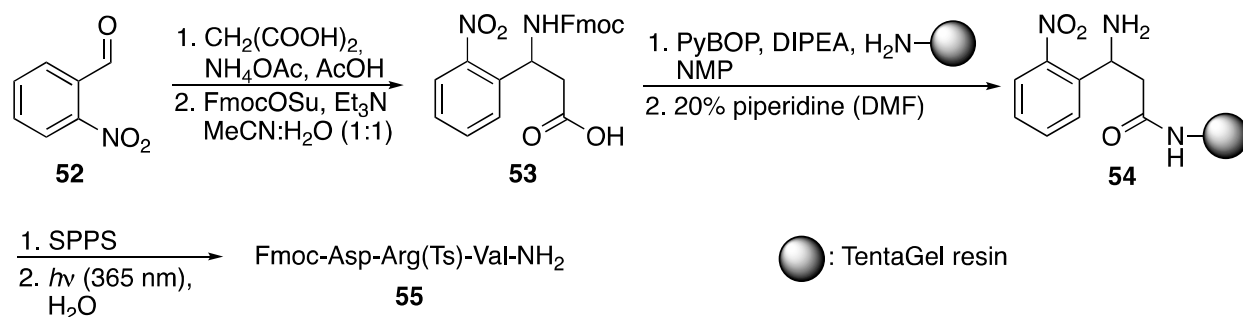
and Gurwara's original *o*-nitrobenzyloxy linker (Scheme 1). Over-nitration destroyed the swelling properties of the resin and prevented the synthesis of longer peptides.

A solution to this issue is to introduce the support itself in the benzylic position instead of a methyl group (Scheme 11).[42] The resin **1** underwent Friedel-Crafts acylation followed by reduction leading to *o*-nitrobenzhydryl resin **48**. With this resin in hand, Ajayaghosh and Pillai synthesized peptides **49**[42] and, after transformation to the amine-functionalized resin **50**,[43] the corresponding amidopeptides **51**. Peptides with up to 10 amino acids were synthesized in good yields.



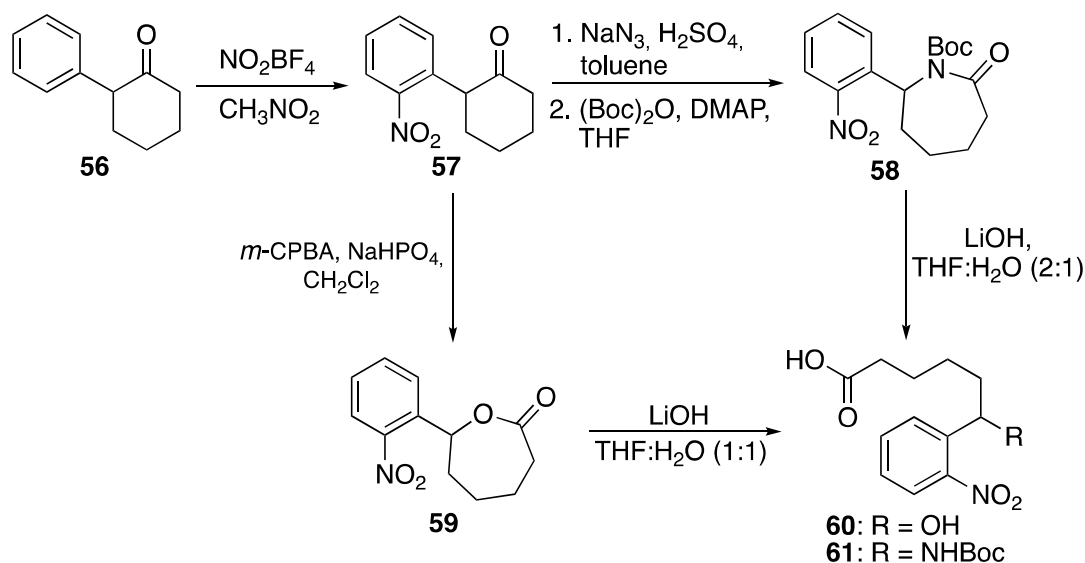
Scheme 11. *o*-Nitrobenzhydryl resin for the synthesis of peptides and amido peptide.

The related 3-amino-3-(2-nitrophenyl)propionyl (ANP) linker **53** was developed by Geysen and coworkers for the synthesis of peptide amides (Scheme 12).[44] Sternson and Schreiber demonstrated a more acid-stable variant established with two methyl groups in the α -position of the carboxylic acid handle.[45] Geysen and coworkers demonstrated the use of an alcohol-functionalized variant of the linker for oligosaccharide synthesis.[46] A longer spacer was incorporated between the support and the photosensitive part to avoid problems with β -elimination and lactonization of the alcohol.



Scheme 12. Synthesis of photolabile ANP resin used for the synthesis of amidepeptide **55**.

An alternate route to similar linkers was designed by Ryba and Harran to access hydroxyl (**60**) and amino-containing variants (**61**), respectively, both from the ketone **56** (Scheme 13).[47]



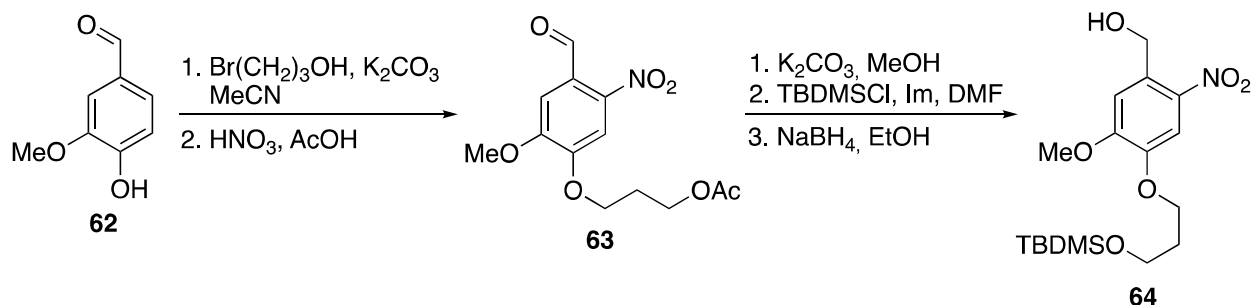
Scheme 13. Synthesis of photolabile ANP type linkers.

o-Nitroveratryl linkers

First introduced by Zehavi and Patchornik,[48] the use of the *o*-nitroveratryl group (4,5-dimethoxy-2-nitrobenzyl) in photolabile linkers was popularized in the mid-1990s by Yoo and Greenberg,[49] and Holmes and Jones.[50]

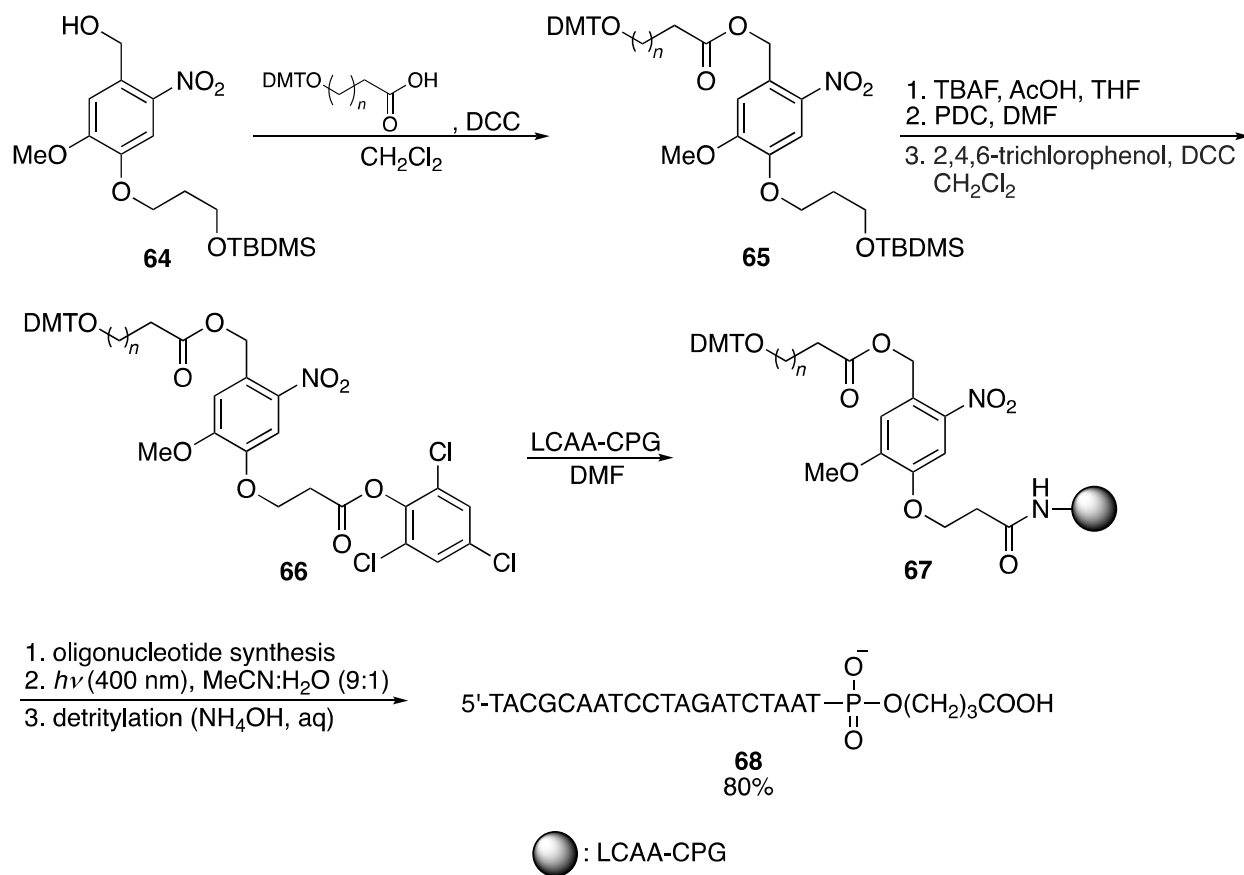
Yoo and Greenberg utilized the linker construct to circumvent some of the issues encountered with the original, unsubstituted *o*-nitrobenzyl linker, namely long photolysis times and low yields

of release.[48] The synthesis of linker **64** started from vanillin (**62**), which after etherification and nitration provided the aldehyde **63**, cf. Scheme 14. After a few functional group interconversions, linker **64** was obtained.[48]



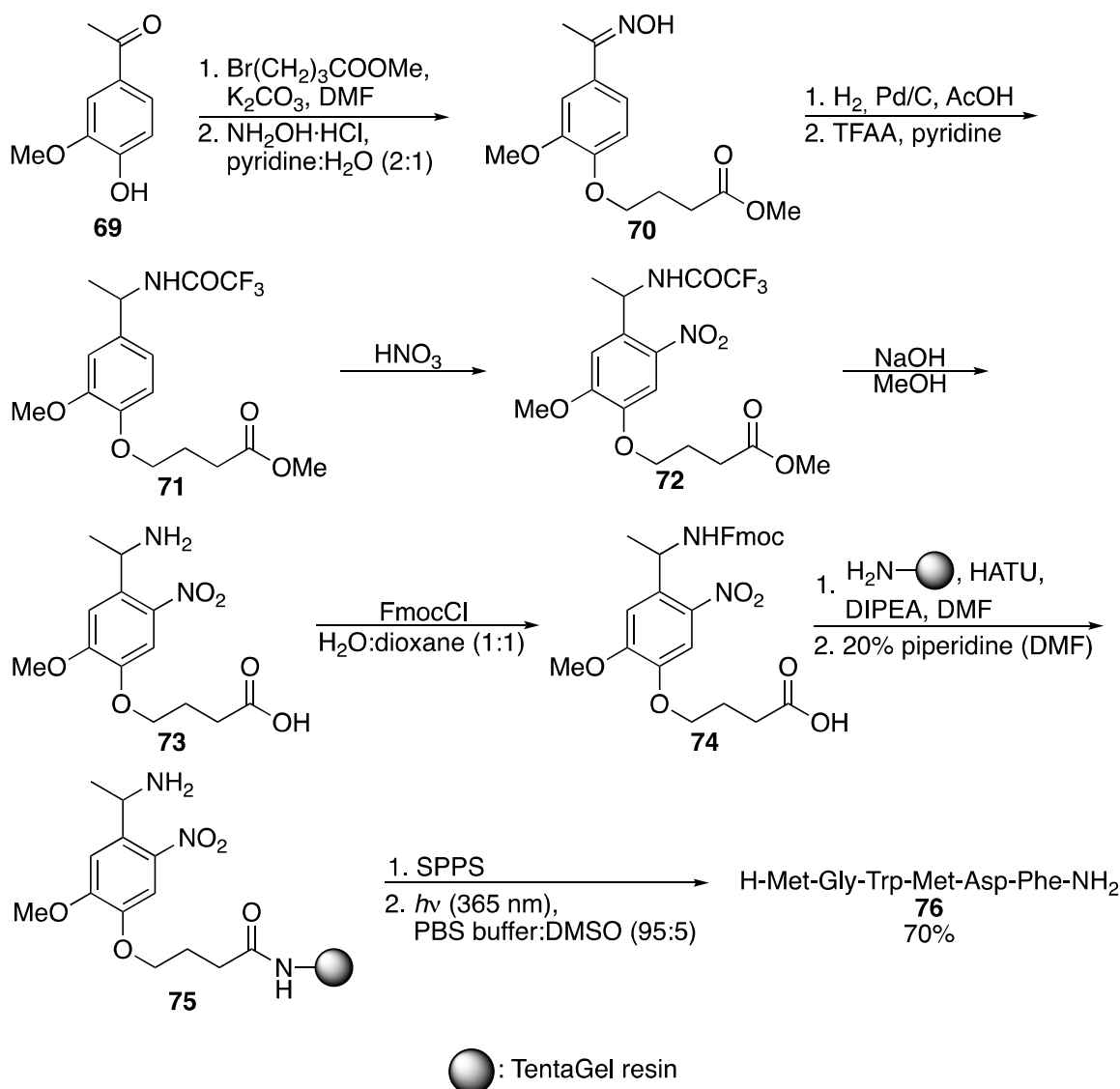
Scheme 14. Synthesis of photolabile *o*-nitroveratryl-based linker.

Linker **64** can readily be installed on a LCAA-CPG support as construct **67**, which was used for the synthesis and release of oligonucleotides in good yields (Scheme 15).[48] Later, it was shown that a more efficient photolysis was achieved when the hydroxyl group of the nucleotide was attached directly to the linker via a carbonate group.[51] Initiated by these studies, this linker has been more broadly implemented in oligonucleotide synthesis.[52]



Scheme 15. Use of photolabile *o*-nitroveratryl-based linker for the synthesis of oligonucleotides.

Holmes and Jones introduced an *o*-nitroveratryl-based amine linker with a methyl group in the α -position.[49] This linker, producing amides upon photolysis, also solved the problem relating to formation of reactive nitroso aldehydes upon photolysis, as described earlier. The synthesis from acetovanillone (**69**) provided linker **74** after a 7-step sequence. Linker **74** was then used for the synthesis of hexapeptide **76** in a yield of 70% (Scheme 16).

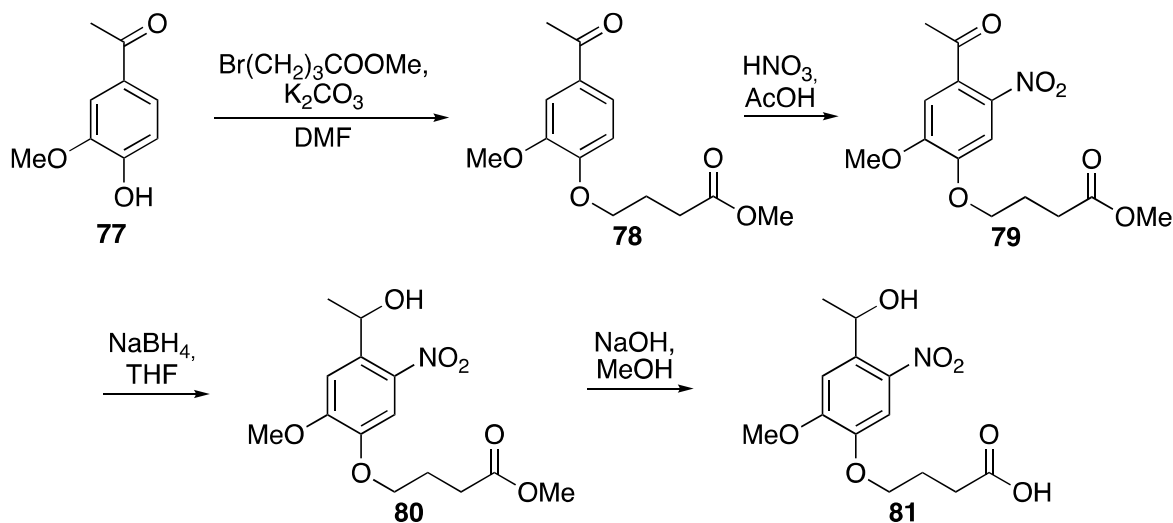


Scheme 16. Synthesis of photolabile amine-containing α -methylated nitroveratryl linker and its use for the synthesis of a hexapeptide.

In 1997, Holmes published a systematic study of the effect of nitroveratryl substituents on the photolysis efficiency.[53] It was shown that the rate of cleavage was 7 to 20 times higher when the *o*-nitrobenzyl ring was substituted with electron-donating alkoxy substituents. It was also shown that an alkyl group in the amine α -position increased the rate by a factor of 3. The length of the spacer chain also influenced the release rate with a slight increase for longer chains. It was

concluded that both the alkoxy substituents on the aromatic ring and the alkyl group in the α -position were beneficial for the photochemical reactivity.

Holmes also developed an alcohol-based version of the linker **74**,[53] which was synthesized analogously to the corresponding amine linker (Scheme 17).

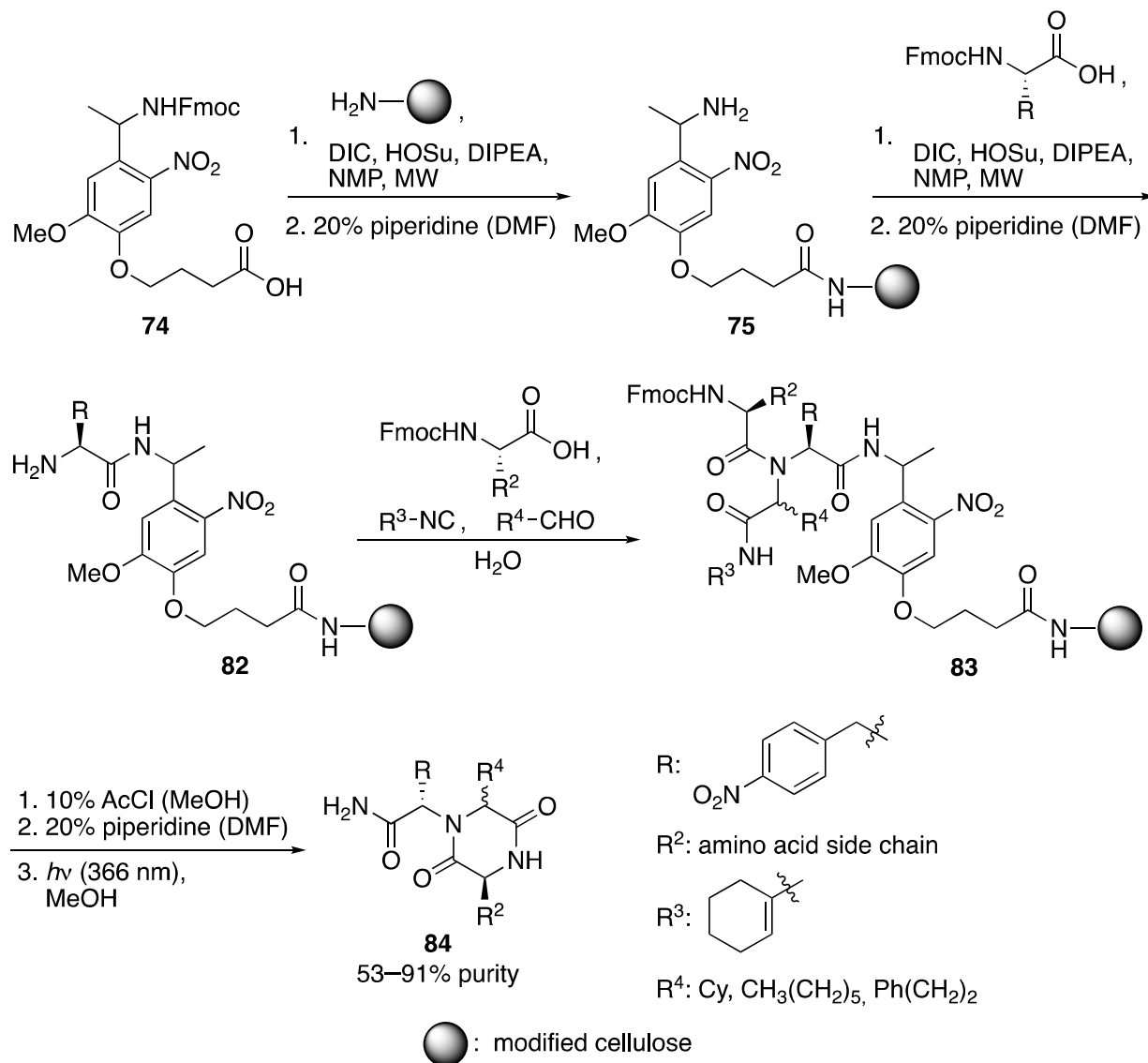


Scheme 17. Synthesis of photolabile alcohol-containing α -methylated nitroveratryl linker

Although, this was a useful route for the synthesis of **81**, problems were encountered in the nitration of **78**, due to contamination with substantial quantities of the ipso substitution of the acetyl group. An improved synthetic pathway to give the ester **80** was developed by Teague.[54] Ipso substitution was substantially reduced, when nitration was performed at low temperature with fuming nitric acid.

The linkers **74** and **81** have been used for numerous applications, and it has been shown that such linkers may be coupled to hydroxyl-based resin without effecting the efficiency of the photolytic cleavage.[55] An *o*-nitroveratryl linker was used by McKeown et al. to develop methods for easing the analysis of solid-phase synthesis reactions by mass spectrometric techniques.[56] Another example is the use of linker **74** by Maddar for the preparation of serine protease mimics.[57] Lin and Blackwell used this linker for the synthesis of a diketopiperazine

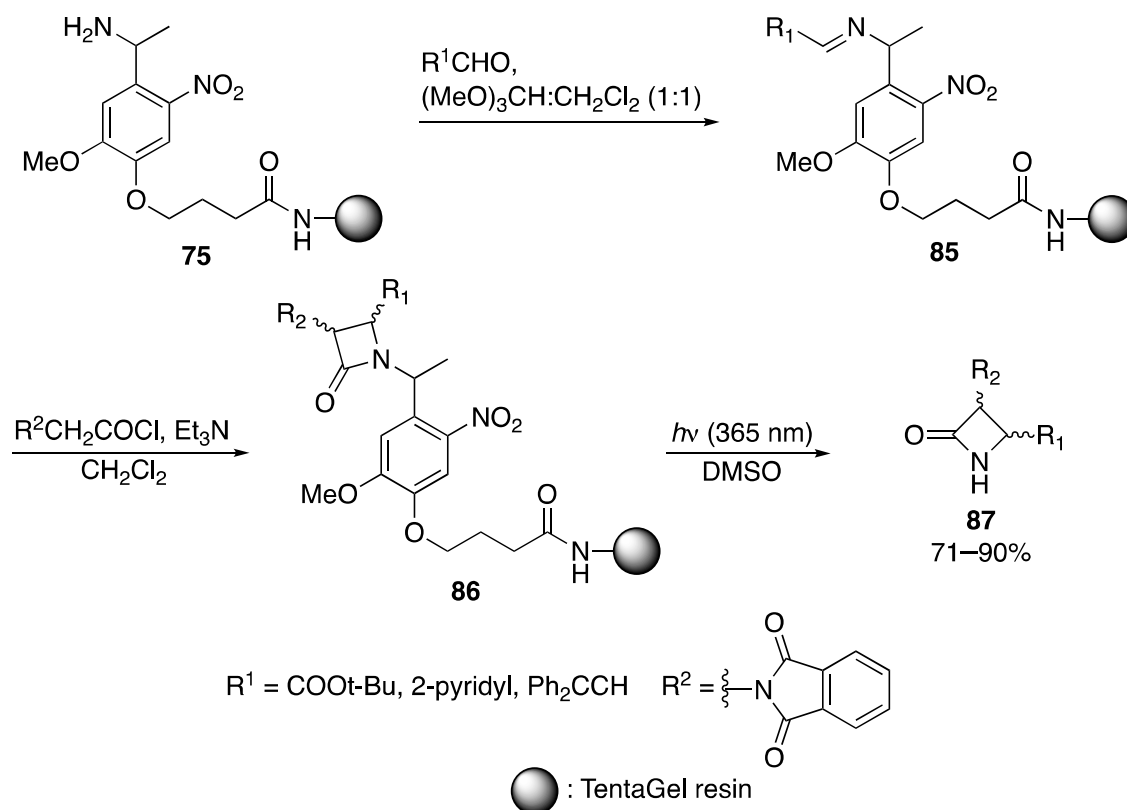
library using the Ugi multi-component reaction (Scheme 18).[58] The linker **74** was attached to a solid support and subsequent coupling of Fmoc-Phe(4-NO₂)-OH and Fmoc deprotection under standard SPPS conditions led to the construct **82**. Ugi reactions, followed by methanolysis and photochemical release afforded diketopiperazines **84** in moderate to good purities.



Scheme 18. *o*-Nitroveratryl linker used in multicomponent reactions by Lin and Blackwell.

Gennari et al. used the linker **74** for the synthesis of a combinatorial library of vinylogous sulfonamidopeptides.[59] This study confirmed the trends encountered by Holmes,[53] namely that *o*-nitroveratryl-based linkers are superior to *o*-nitrobenzyl linkers, due to the beneficial effects of alkoxy-substituents and the presence of a α -methyl group.

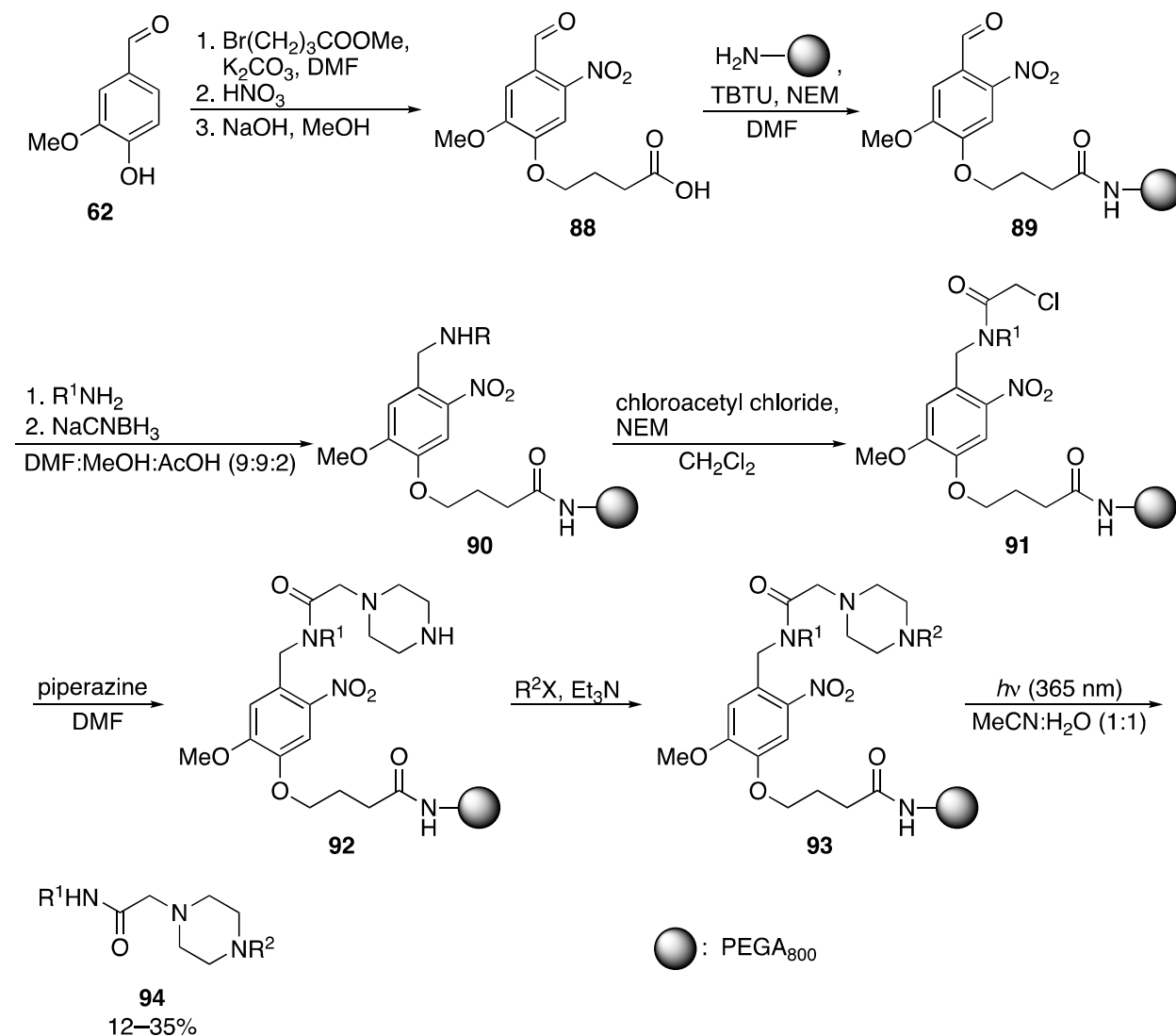
Another example is the synthesis of 3,4-disubstituted β -lactams by Gallop and coworkers (Scheme 19).[60] The construct **75** was condensed with an aldehyde to form imines **85** which then underwent a [2+2] cycloaddition with a ketene to provide **86**. Finally, photolysis led to β -lactams **87** in good yields (Scheme 19).[60].



Scheme 19. α -Methylated nitroveratryl linker used in the synthesis of β -lactams.

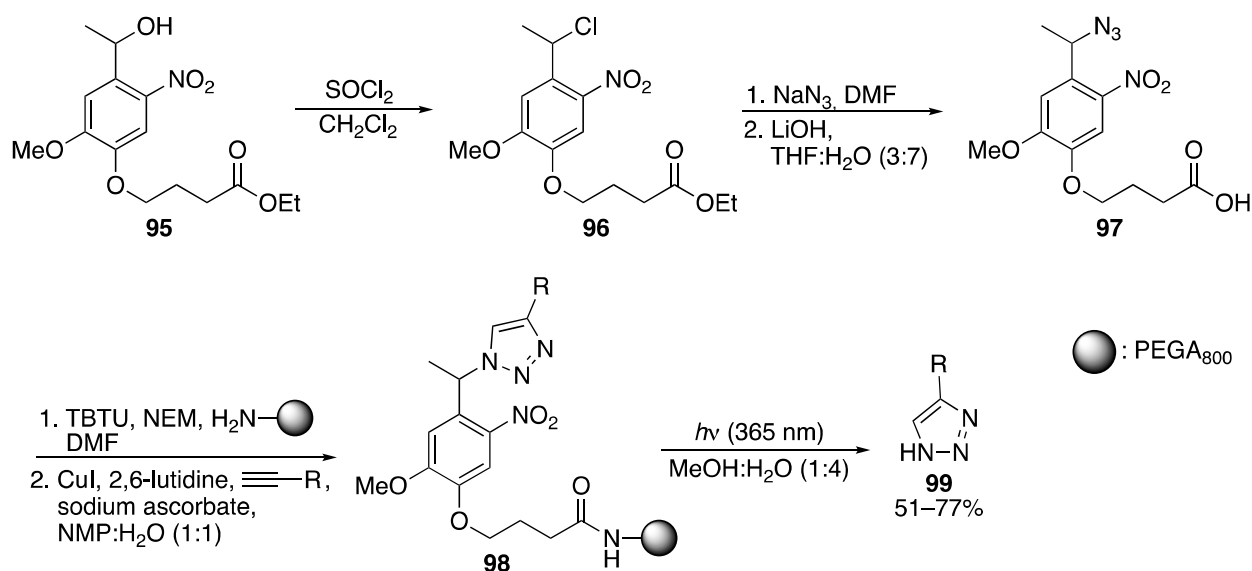
Minkwitz and Meldal used the aldehyde linker **88**, related to **74**, for the synthesis of a small library of arylpiperazine melanocortin subtype-4 agonists.[61] The linker **88** was synthesized and

attached to the solid support (Scheme 20). Construct **89** was then subjected to reductive amination and subsequent acylation of the formed amine afforded **91**. Finally, two alkylation steps and photolytic cleavage provided arylpiperazines **94**.



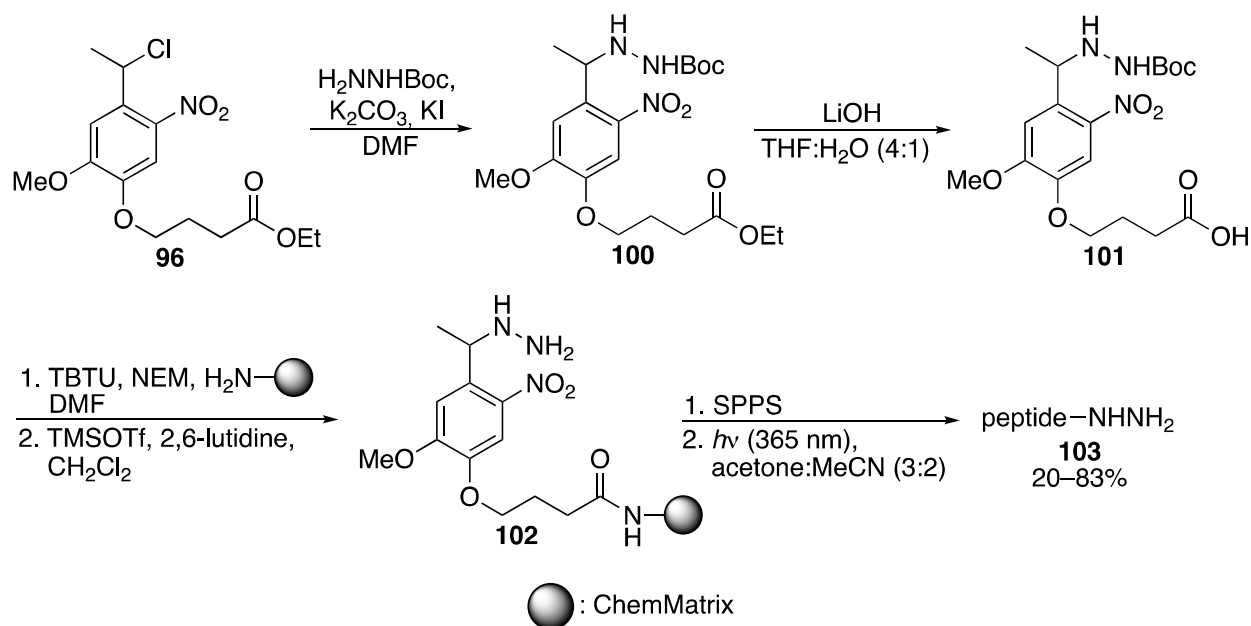
Scheme 20. Photolabile linker used for the synthesis of melanocortin subtype-4 agonists.

Qvortrup and Nielsen introduced an azido-functionalized linker based on the *o*-nitroveratryl group for the synthesis of 1,2,3-triazoles,[62] cf. Scheme 21.



Scheme 21. Photolabile nitroveratryl-based azido-linker used for the synthesis of 1,2,3-triazoles.

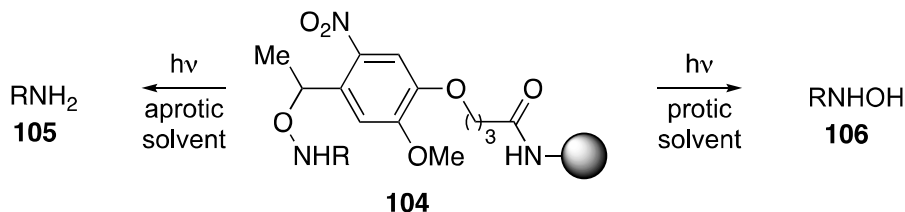
The alcohol **95** was synthesized through procedures adapted from Holmes and co-workers (Scheme 17). The alcohol was then subjected to SOCl_2 and after substitution with NaN_3 and ester hydrolysis with LiOH , the azido linker **97** was obtained. Attachment to the solid support and subsequent copper(I)-catalyzed azide–alkyne cycloaddition led to the construct **98**, which after photolytic cleavage provided 1,2,3-triazoles **99** in good yields. Nielsen and coworkers later introduced a hydrazine linker.[63] Starting from chloride **96**, the linker **100** was synthesized in two steps, and following attachment to the solid support, SPPS and photolytic cleavage provided peptide hydrazides **103** in yields of 21–83% (Scheme 22).



Scheme 22. Photolabile nitroveratryl-based linker used for the synthesis of peptide hydrazides.

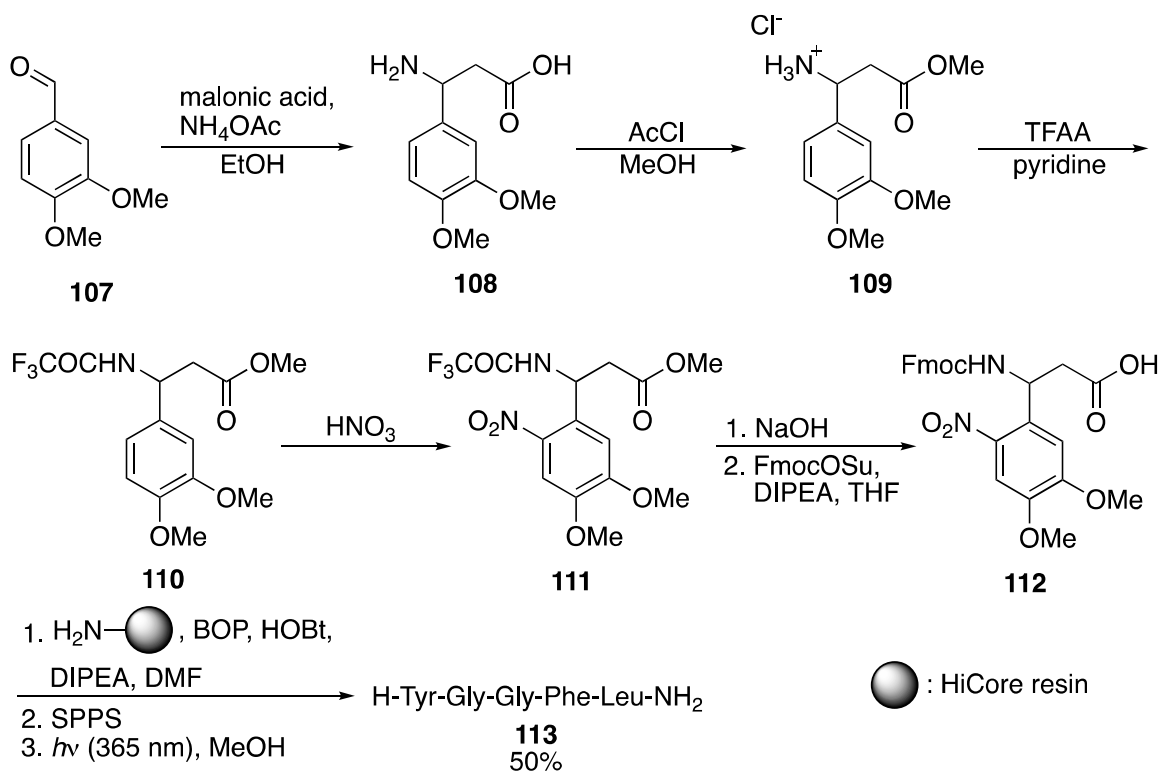
The construct **101** could also be utilized for the solid-phase synthesis of dihydropyrano[2,3-c]pyrazoles.[63]

Recently, Qvortrup et al. introduced a photolabile nitroveratryl-based linker **104** for the synthesis and release of hydroxamic acids.[64, 65] Furthermore, they showed, that this linker unit may, uniquely, function as a ‘bidetachable’ system.[64] By simply varying the reaction solvent, the photolysis could be controlled to provide either C-O or C-N bond cleavage, which allowed for controlled release of the hydroxamates **106** or carboxamides **105**, respectively (Scheme 23).



Scheme 23. Bi-detachable linker: Solvent-controlled chemoselectivity of photolysis to provide the hydroxamate or carboxamide, respectively.

Another recent example is the linker developed by Lee and co-workers.[66] This linker is tethered to the solid support through the α -position. Starting from veratraldehyde (**107**) the linker **112** was synthesized in six steps (Scheme 24).



Scheme 24. Synthesis of Leu-enkephalin amide using photolabile linker **112**.

After coupling to the solid support, standard SPPS and photolytic cleavage provided Leu-enkephalin amide (**113**) in a yield of 50%.

Phenacyl linkers

Another useful photo-cleavable linker is based on the phenacyl [PhC(O)CH_2 -] group. The aryl carbonyl undergoes $n \rightarrow \pi^*$ or $\pi \rightarrow \pi^*$ excitation upon UV radiation (300-350 nm), followed by hydrogen atom abstraction from solvent and bond scission to cleave the linker (Figure 3).[67]

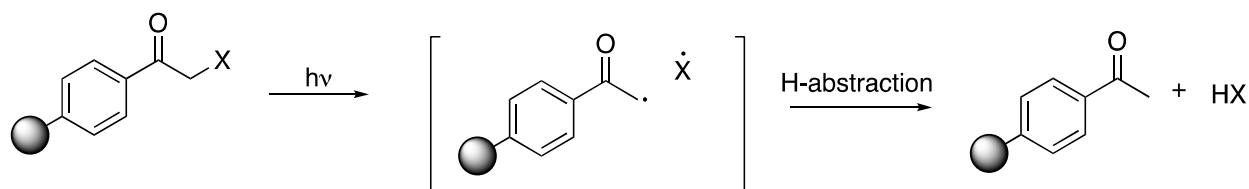
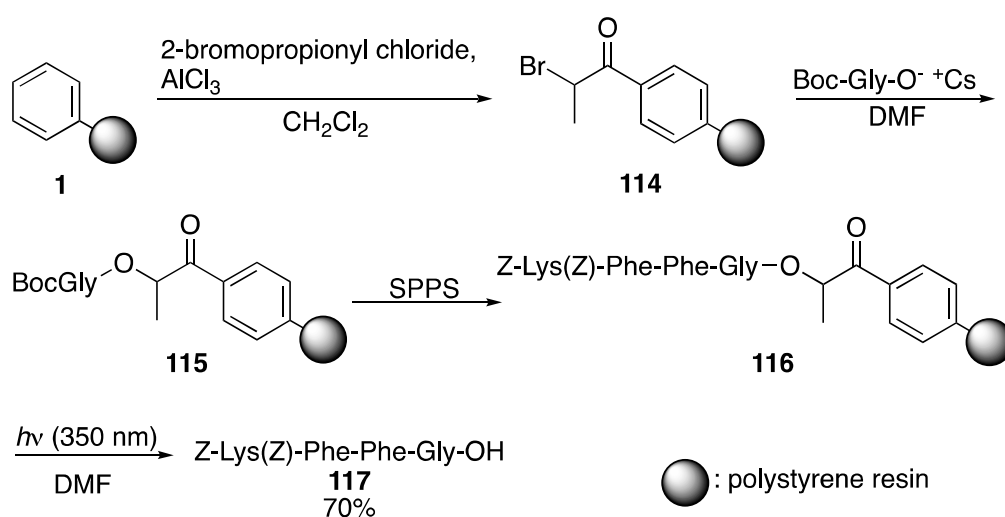


Figure 3. Photochemistry of aromatic ketones that release a leaving group (X).

The phenacyl group was explored in pioneering studies on photolabile linkers by Wang.[68] The resin **1** was subjected to Friedel-Crafts acylation with 2-bromopropionyl chloride, and the first amino acid was *O*-alkylated to provide construct **115**, cf. Scheme 25. Subsequent standard SPPS and final photolysis released the peptide **117** in 70% yield.

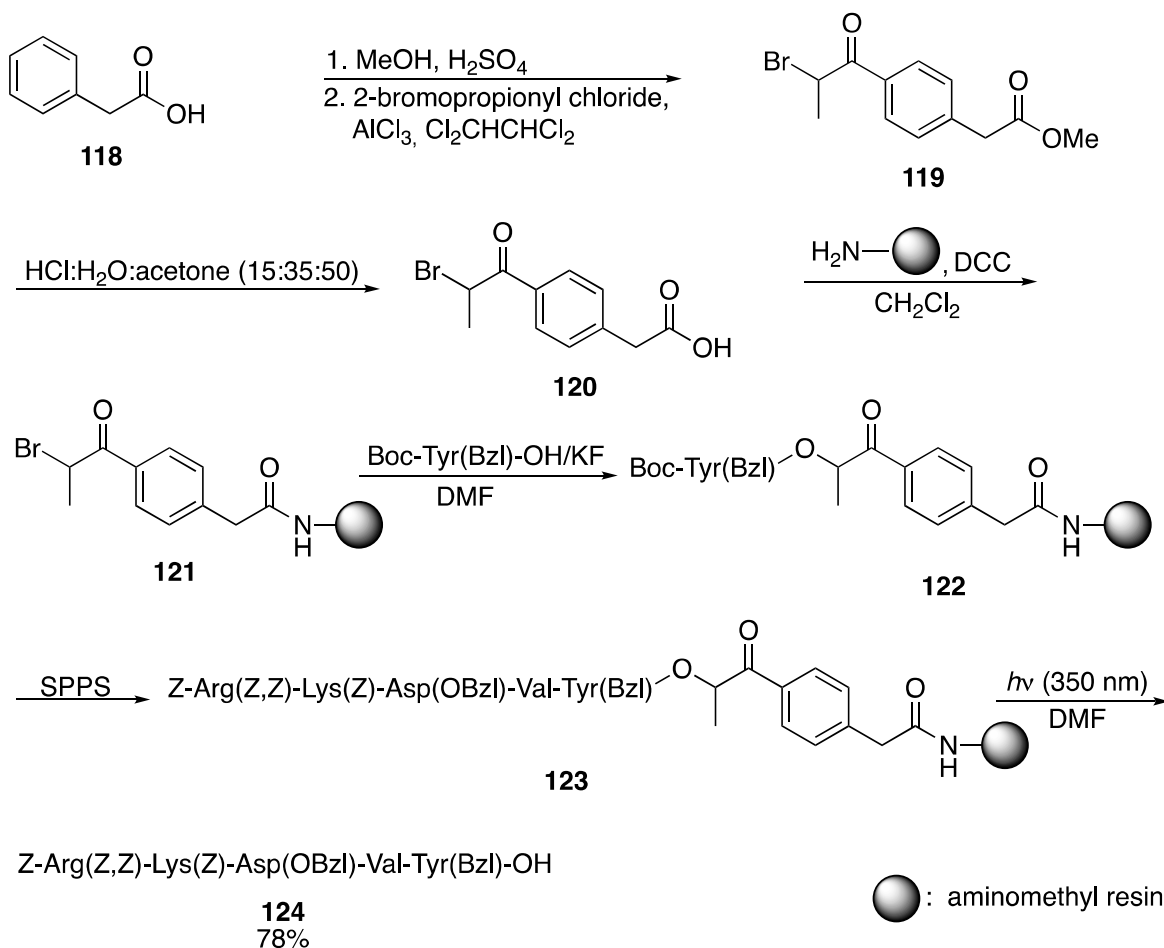


Scheme 25. Synthesis of a phenacyl photolabile handle and its use in peptide synthesis.

A phenacyl linker was also used by Tam to produce resins cleavable under both photolytic, basic or nucleophilic conditions.[12, 13]

Tjoeng and Heavner developed a non-integral phenacyl linker system,[69] which was synthesized in solution and coupled to the resin, to avoid the direct derivatization of the resin as described by Wang. Initial esterification of phenylacetic acid (**118**) and subsequent Friedel-

Crafts acylation led to the methyl ester **119**, which upon simple ester hydrolysis led to the phenacyl linker **120** (Scheme 26). With **120** in hand, attachment to the resin and coupling of the first amino acid, followed by standard SPPS led to the peptide **124** in 78% yield after photolytic release.[68]

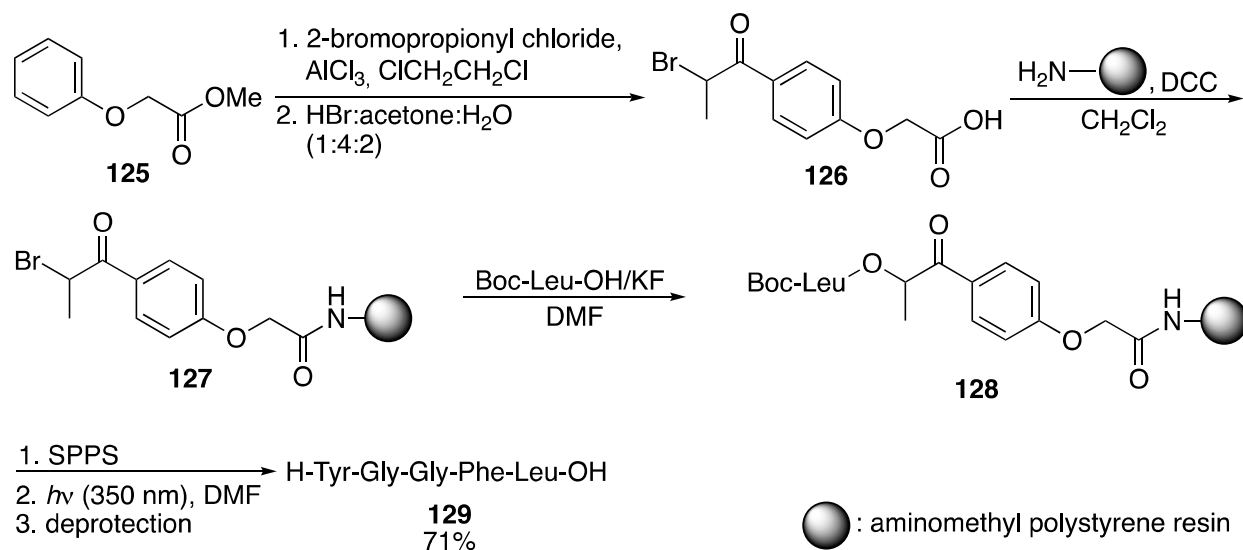


Scheme 26. Synthesis of phenacyl photolabile linker **120** and its use for the synthesis of thymopoietin II fragments.

p-Alkoxyphenacyl linkers

Introduced by Bellof and Mutter,[70] the *p*-alkoxyphenacyl linker is structurally similar to the phenacyl linker, but the presence of an electron donating alkoxy group in the para-position induces a bathochromic shift, making the photolysis at 350 nm much more efficient, thus

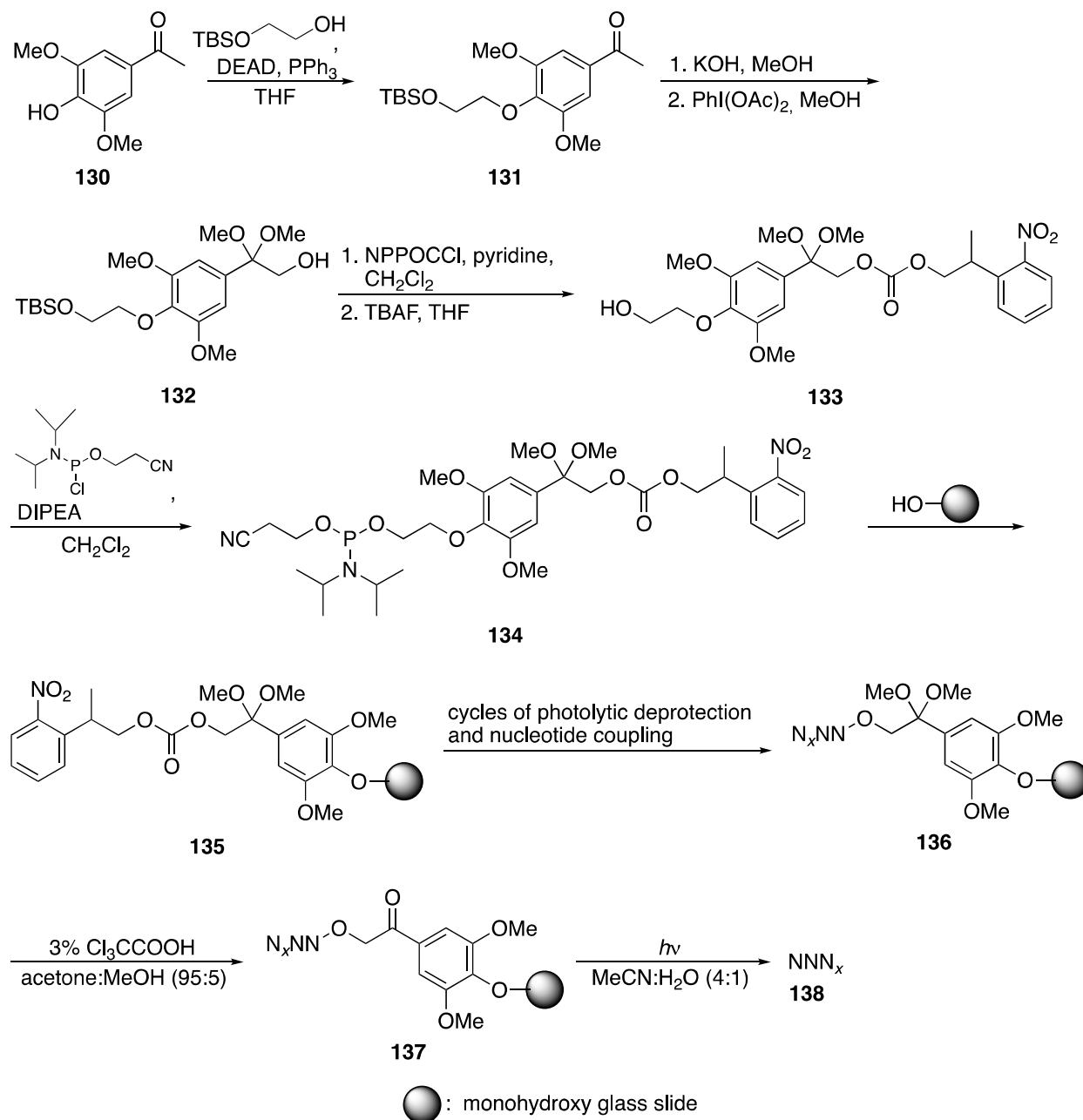
shortening the irradiation times. The synthesis started from methyl phenoxyacetate (**125**), which after Friedel-Crafts acylation and ester hydrolysis provided the bromide **126** (Scheme 27). Coupling to the resin followed by attachment of the first amino acid, either in the presence of KF or as its cesium salt, led to **128**. Conventional SPPS, photolysis and deprotection led to the peptide **129** in 71% yield.



Scheme 27. Synthesis of a *p*-alkoxyphenacyl photolabile linker for peptide synthesis.

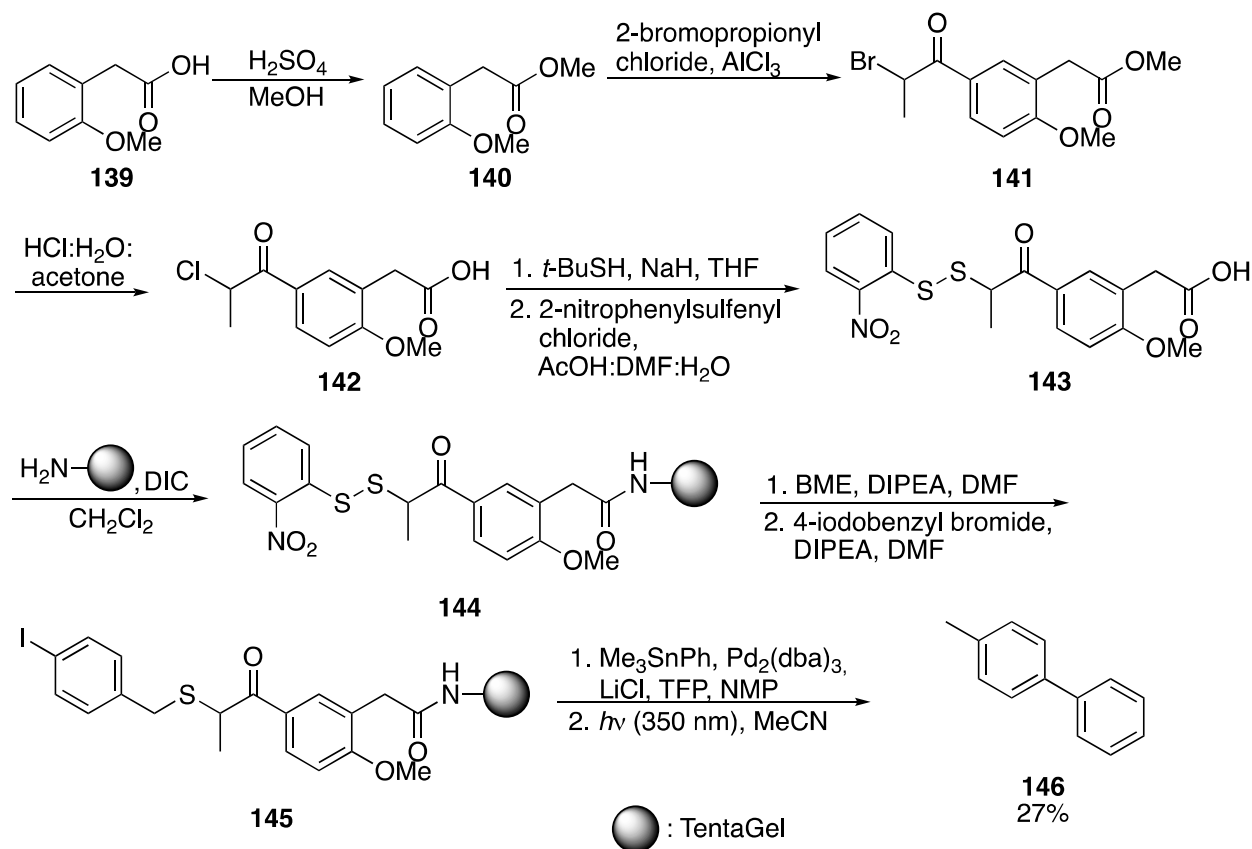
Gauthier and co-workers used the same linker for the synthesis of octapeptides in good yield (>85%), as illustrated by the efficient synthesis of mammalian glucagon.[71] Belshaw and co-workers developed a safety-catch photolabile linker based on the *p*-alkoxyphenacyl moiety.[72] The photolability of the linker was masked to enable the use of photolabile protecting groups in light-directed synthesis of oligonucleotides. The linker synthesis started from acetophenone **130**, (Scheme 28). A Mitsunobu reaction led to **131**, which after acetal formation and methyl oxidation provided the alcohol **132**. Protection with the photolabile NPPOC group followed by alcohol deprotection gave **133**, which was then prepared for attachment to the solid support by treatment with 2-cyanoethyl diisopropylchlorophosphoramidite resulting in the linker **134**. Linker

134 was attached to the solid support and iterative cycles of photolytic deprotection and coupling of nucleotides bearing a photocleavable protecting group furnished construct **136**. Mild acidic hydrolysis then activated the linker for photolytic cleavage and final photolysis provided the DNA chain **138**.



Scheme 28. Synthesis and use of a safety-catch photolabile linker for light directed synthesis and release of oligonucleotides (NNN_x = DNA chain).

Sucholeiki introduced a traceless thioether *p*-methoxyphenacyl linker for use in Stille cross-couplings.[73, 74] Methylation of **139**, Friedel-Crafts acylation and halogen exchange led to the chloride **142** (Scheme 29).



Scheme 29. Synthesis and use of a traceless thioether *p*-methoxyphenacyl linker in Stille cross-coupling reactions.

Substitution and disulfide formation then furnished the linker **143**, which after attachment to the solid support and disulfide cleavage was reacted with 4-iodobenzyl bromide to provide construct **145**. Pd-catalyzed Stille cross-coupling and subsequent photolysis led to the biaryl **146** in a yield of 27%.

Benzoin linkers

The photolysis of 3',5'-dialkoxybenzoin can be rationalized as a diradical process starting from an $n \rightarrow \pi^*$ transition. A charge transfer interaction of the electron-rich dialkoxyphenyl moiety with the electron deficient oxygen of an $n \rightarrow \pi^*$ singlet excited ketone form an intramolecular exciplex, which after rearrangement, leads to release of the product and formation of the benzofuran (Figure 4).

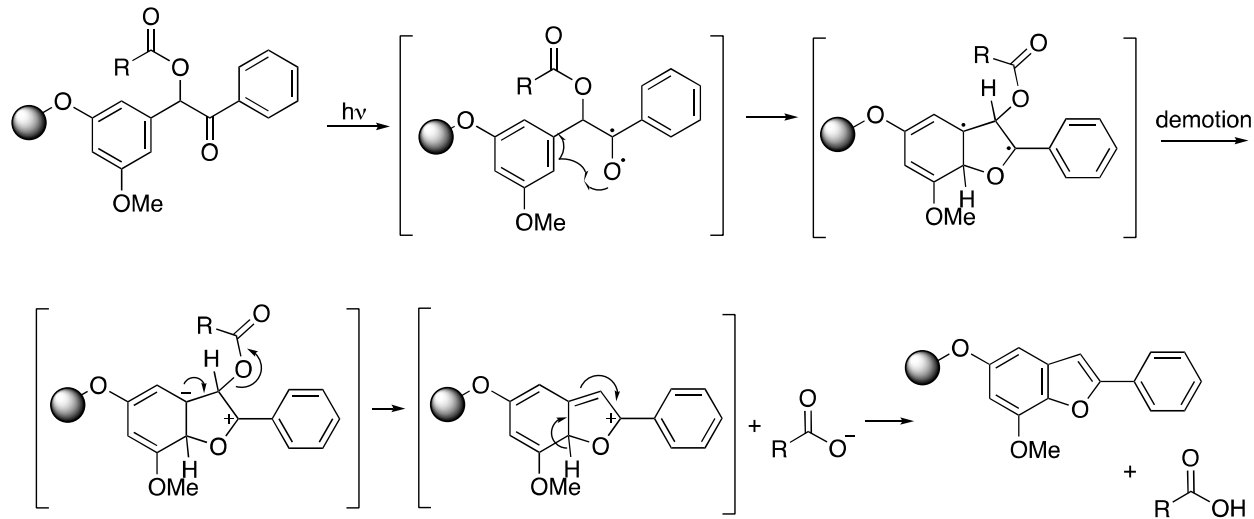
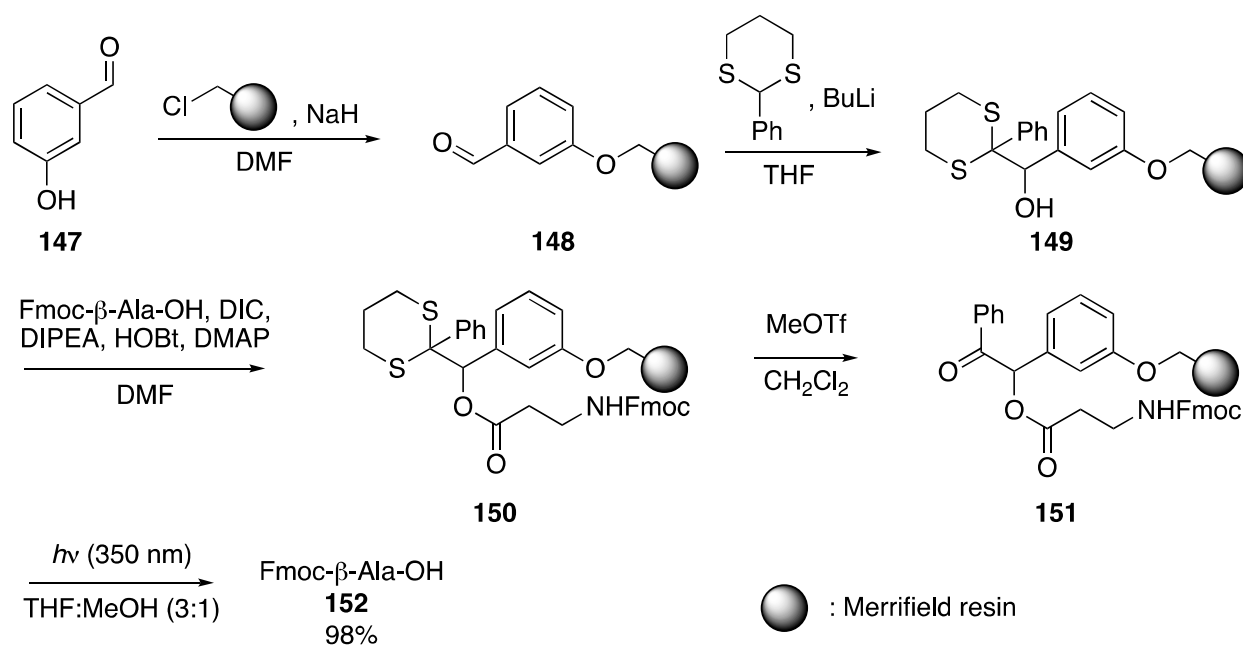


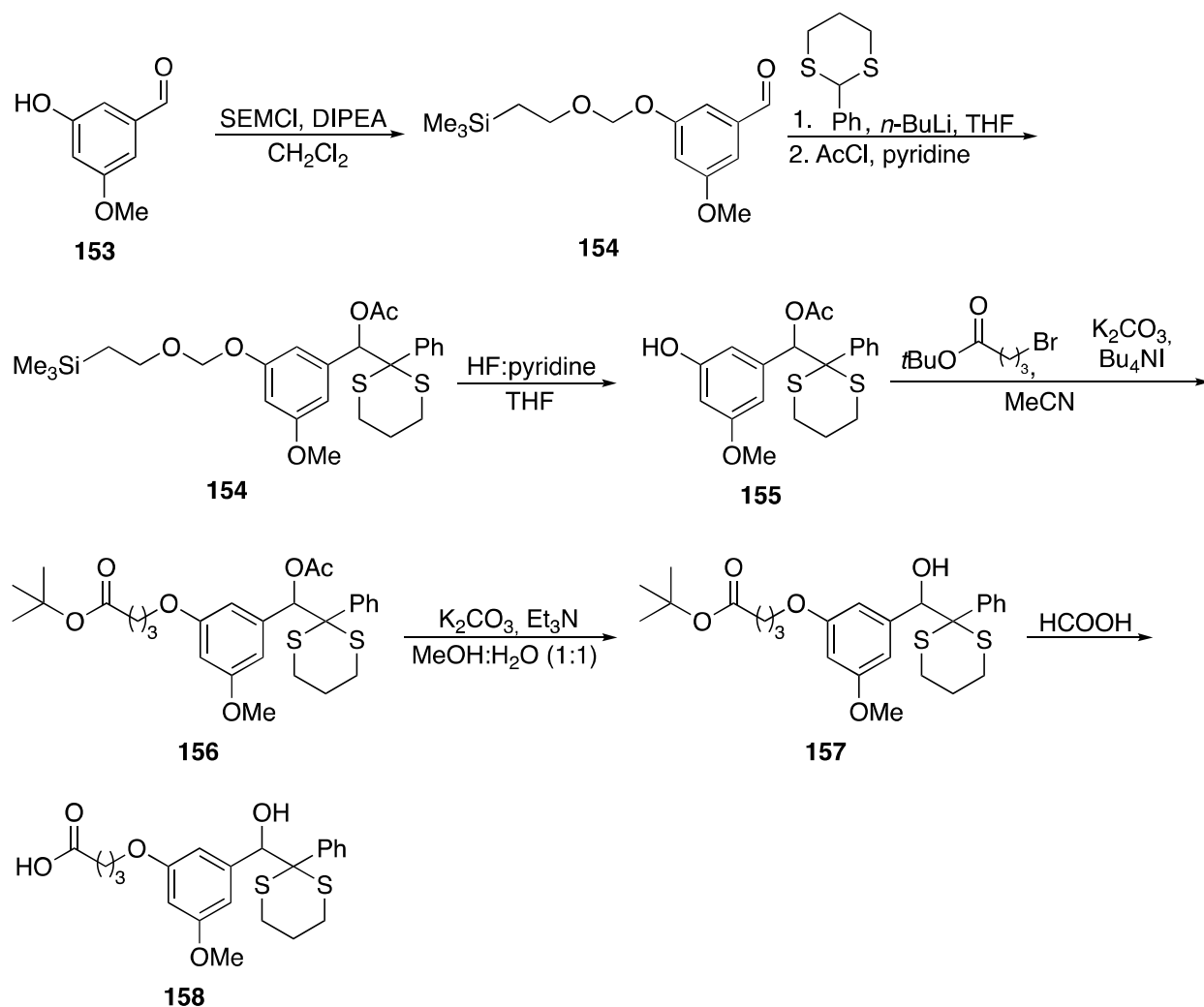
Figure 4. Photoinduced cleavage of a Benzoin linker.

Rock and Chan developed a benzoin safety-catch linker for the purpose of binding two molecules together in solution.[75] This was later exploited for solid-phase synthesis by Balasubramanian and co-workers.[76, 77] The synthesis started from 3-hydroxybenzaldehyde (**147**) which was coupled to the resin and reacted with 2-phenyl-1,3-dithiane to form a dithioacetal, which effectively functioned as a safety-catch moiety that can be triggered upon hydrolysis (Scheme 30). Fmoc- β -Ala-OH was then coupled to the resin to give **150**, which upon hydrolysis of the dithioacetal and photolysis released the amino acid **152** in excellent yields. Good to excellent yields (75–97%) were obtained using this strategy with other carboxylic acids.



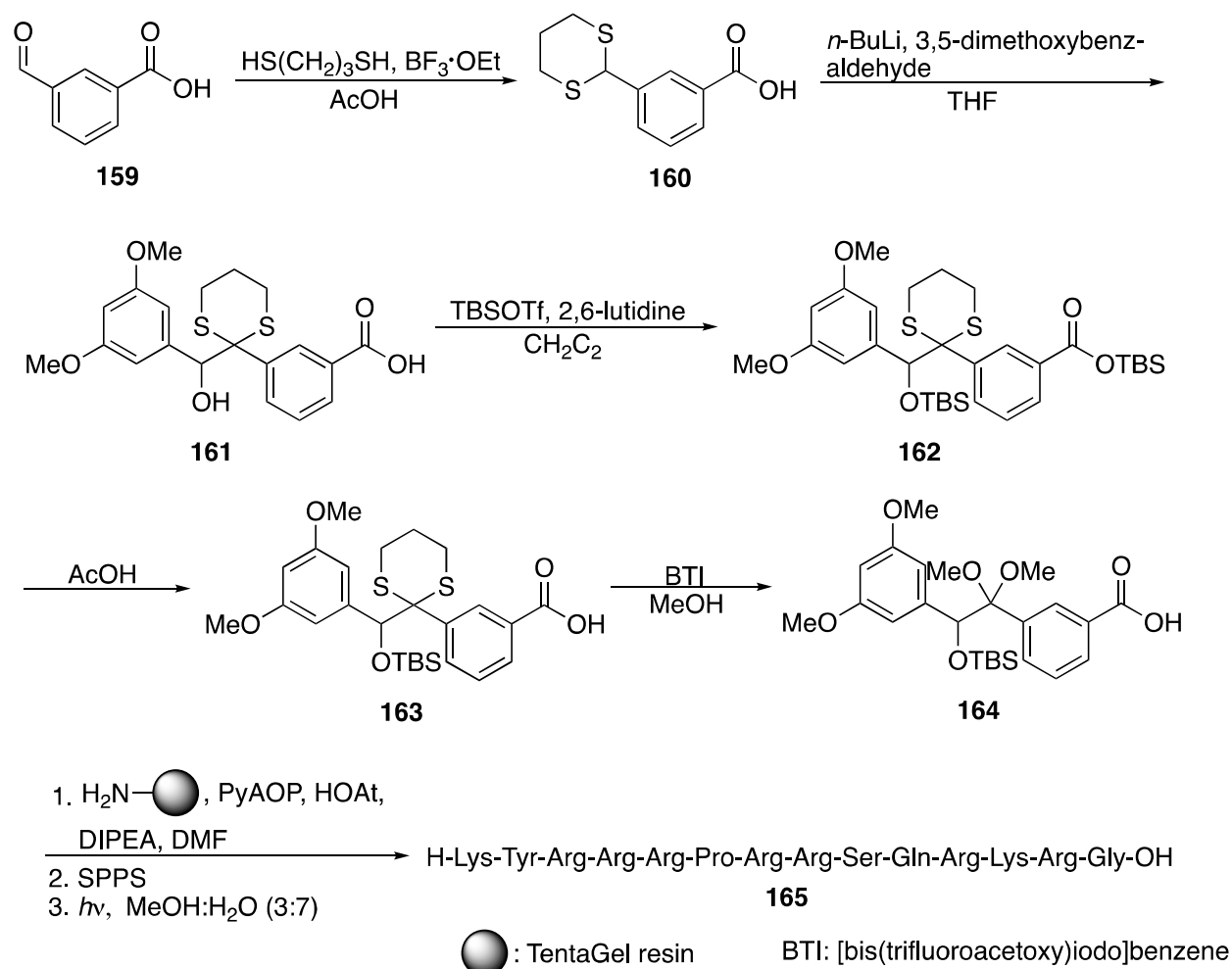
Scheme 30. Use of photolabile benzoin safety-catch linker in solid-phase synthesis.

Later Balasubramanian and co-workers developed a second-generation, non-integral benzoin linker.[78] The linker was prepared with a carboxylic acid as anchoring point to exploit the wide range of amine functionalized resins available, notably with a long tether, as this had previously been shown to be advantageous for *o*-nitroveratryl linkers. The synthesis started from aldehyde **153** and a six-step sequence led to the linker **158**, cf. Scheme 31.



Scheme 31. Synthesis of a photolabile benzoin linker with a carboxylic acid anchor.

To address the often challenging dithioacetal removal, Copley and coworkers developed a new benzoin linker in which the carbonyl group was protected as a dimethyl ketal.[79] The dimethyl ketal moiety is stable towards common solid-phase esterification and amide bond formation reaction conditions, yet easily removable with 3% TFA. The synthesis of the linker started from 3-formylbenzoic acid (**159**), followed by a four-step sequence to provide dithioacetal **163** (Scheme 32). Finally, the dithioacetal functionality was replaced with a dimethyl ketal to provide linker **164**. Attachment to the solid support, subsequent SPPS and photolysis released the tetradecapeptide **165**.



Scheme 32. Synthesis and use of a photolabile dimethyl ketal benzoin linker for peptide synthesis.

Pivaloyl linkers

Pivaloyl derivatives fragment by a Norrish type I mechanism, releasing carboxylic acids or alcohols (Figure 5). The side-products, carbon monoxide and isobutene, are all volatile, making purification simple. However, the process requires the use of a relatively short wavelength source (280–340 nm), which may cause side reactions when applied to more fragile biomolecules.

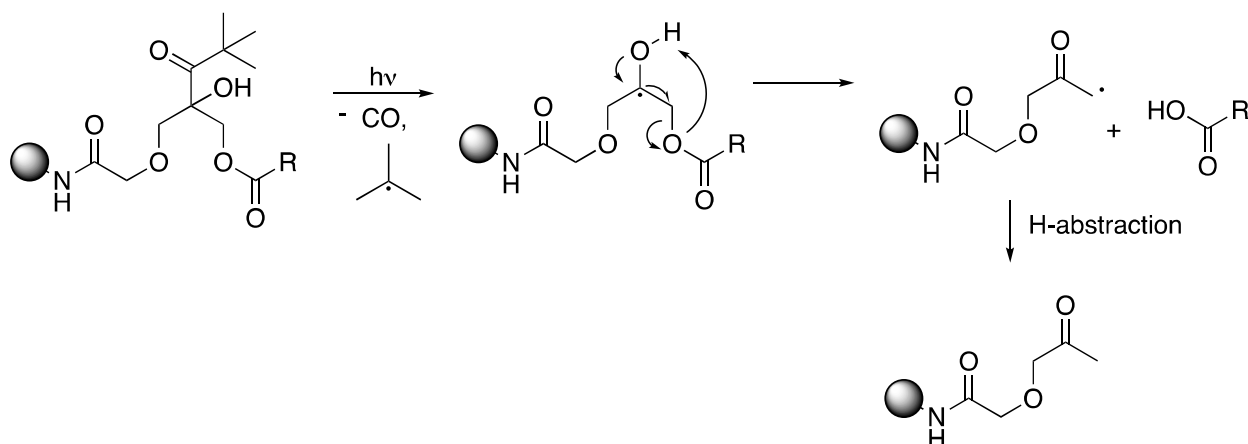
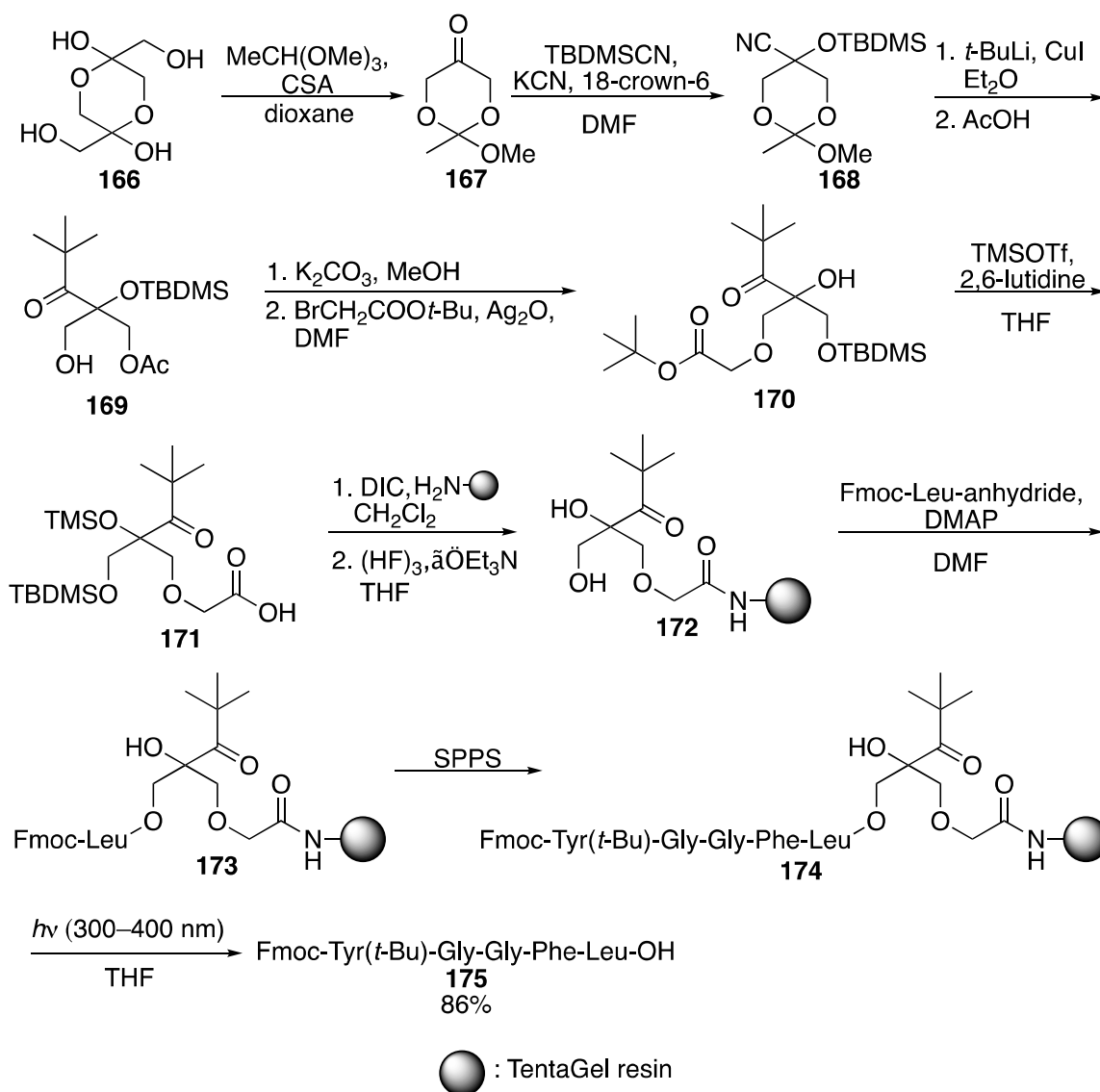


Figure 5. Photolysis of a pivaloyl-based linker.

Peukert and Giese developed a photolabile linker based on the pivaloyl group.[80] The synthesis started from 1,3-dihydroxyacetone dimer (**166**) and a six-step sequence gave the linker **171** in an overall yield of 40% (Scheme 32). The linker was then coupled to the solid support and following attachment of the first amino acid, standard SPPS provided construct **174**, which upon photolysis released the peptide **175** in a yield of 86%.

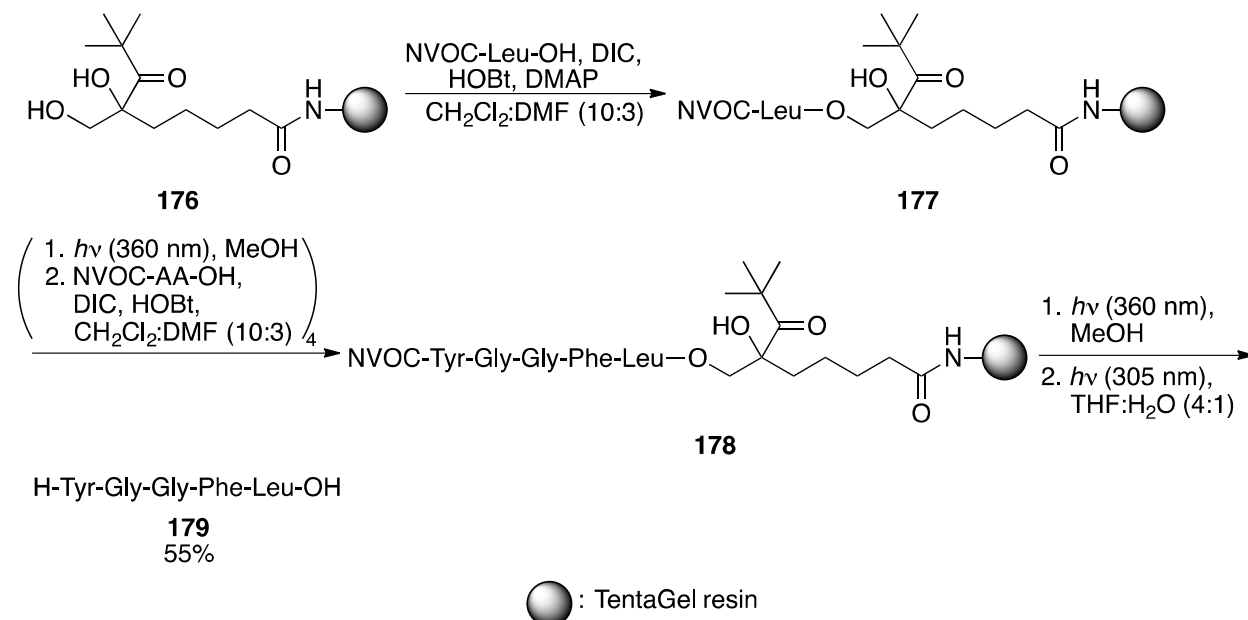


Scheme 33. Synthesis and use of a pivaloyl-based photolabile linker.

The linker **171** also proved useful for the generation and release of compounds relying on Pd-catalyzed Stille and Suzuki cross-coupling, and epoxidation reactions.[80] Later, a modified linker enabled the release of alcohols upon photolysis.[81]

Bochet and coworkers exploited the property of the pivaloyl linker, used in the synthesis of Leu-Enkephalin, as being stable at longer wavelengths, thus enabling the use of photolabile protection groups with different chromatic lability.[82] The synthesis started with the coupling

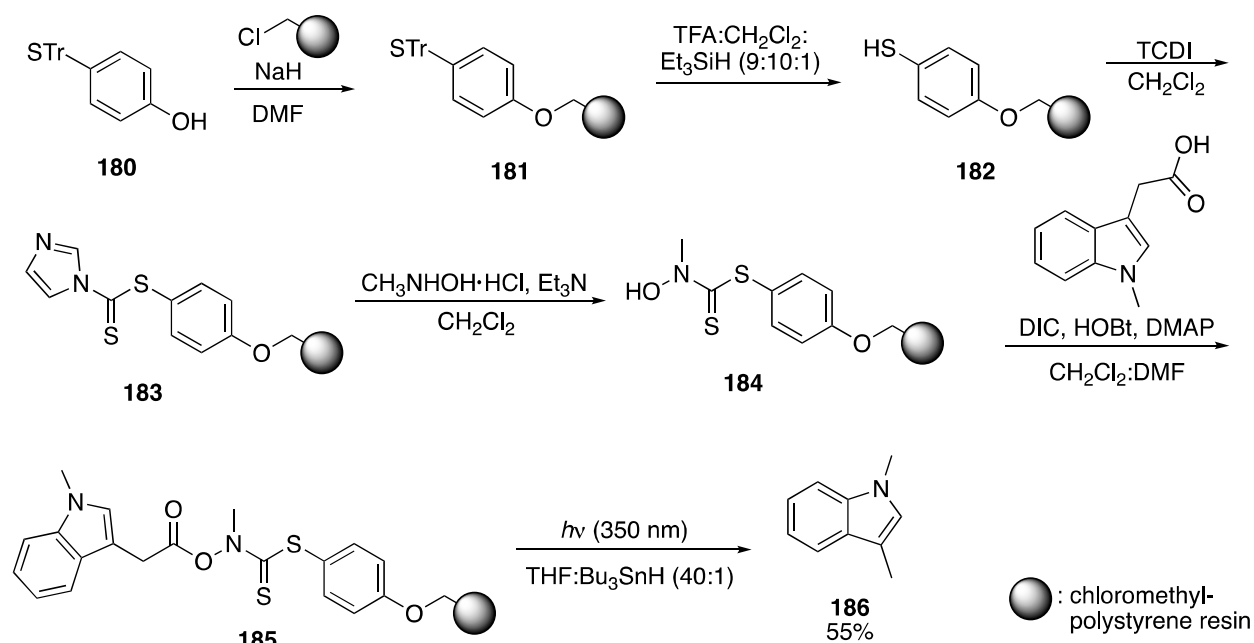
of NVOC-Leu-OH to the linker resin **176** under standard conditions (Scheme 34). Four cycles of photolytic deprotection (360 nm) and amino acid coupling reactions furnished construct **178**. Finally deprotection and photolytic cleavage from the resin (305 nm) released Leu-enkephalin **179** in a yield of 55%.



Scheme 34. Synthesis of Leu-Enkephalin utilizing both a photolabile linker and photolabile protecting groups.

Other photolabile linkers

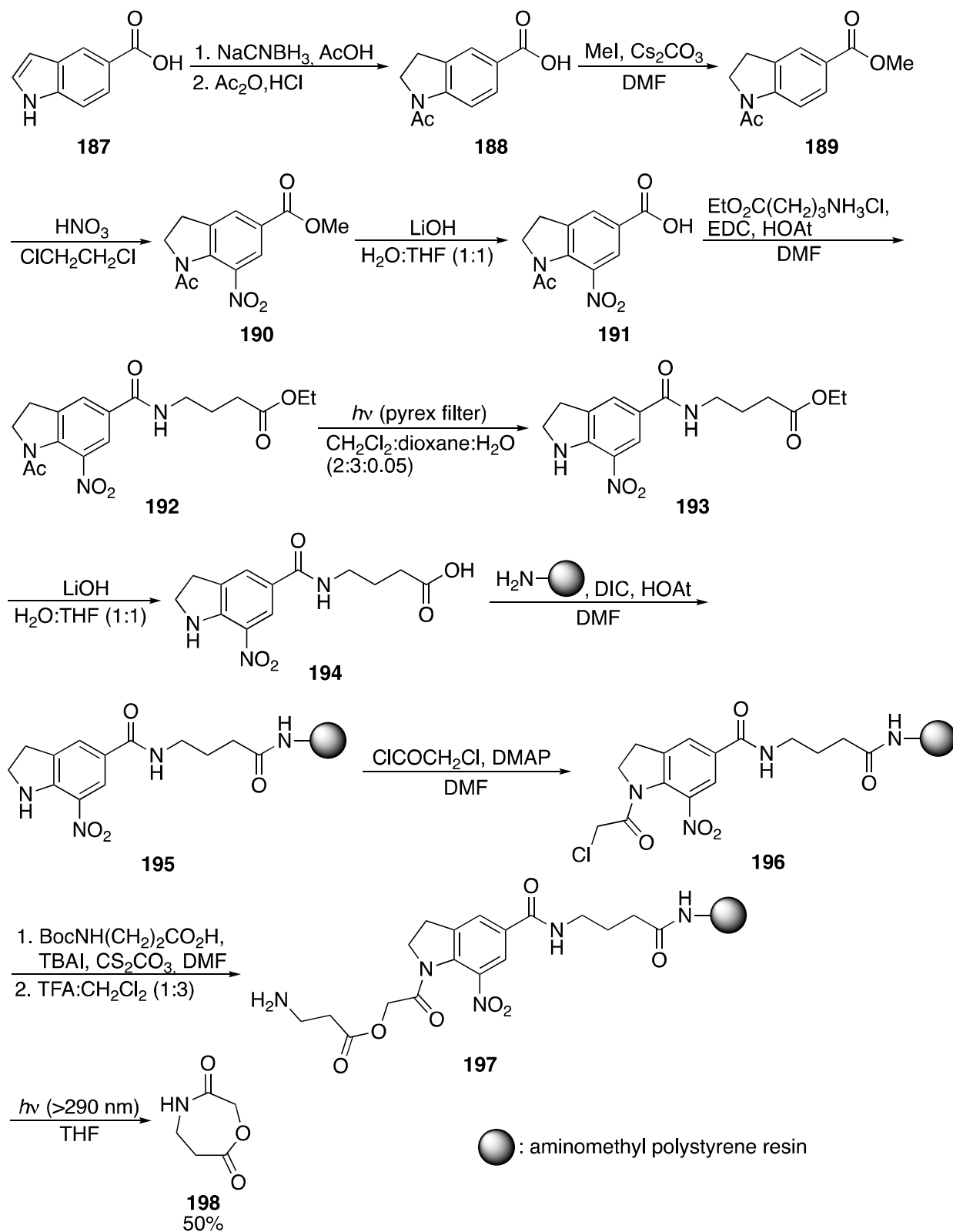
Routledge and co-workers introduced a traceless photolabile linker based on thiohydroxamic acid.[83] Starting with attachment of trityl-protected 4-hydroxymercaptophenol **180** to the solid support, the linker construct **184** was prepared in four steps (Scheme 35).



Scheme 35. Synthesis and use of a traceless photolabile linker.

Then *N*-methylindole-3-acetic acid was attached, and photolysis yielded 1,3-dimethylindole **186** in a yield of 55%. This method, however, suffers from the need of a hydrogen-donor, such as a toxic tin hydride species to facilitate release from the solid support, whereas conventional photolysis normally can be achieved under mild and neutral conditions.

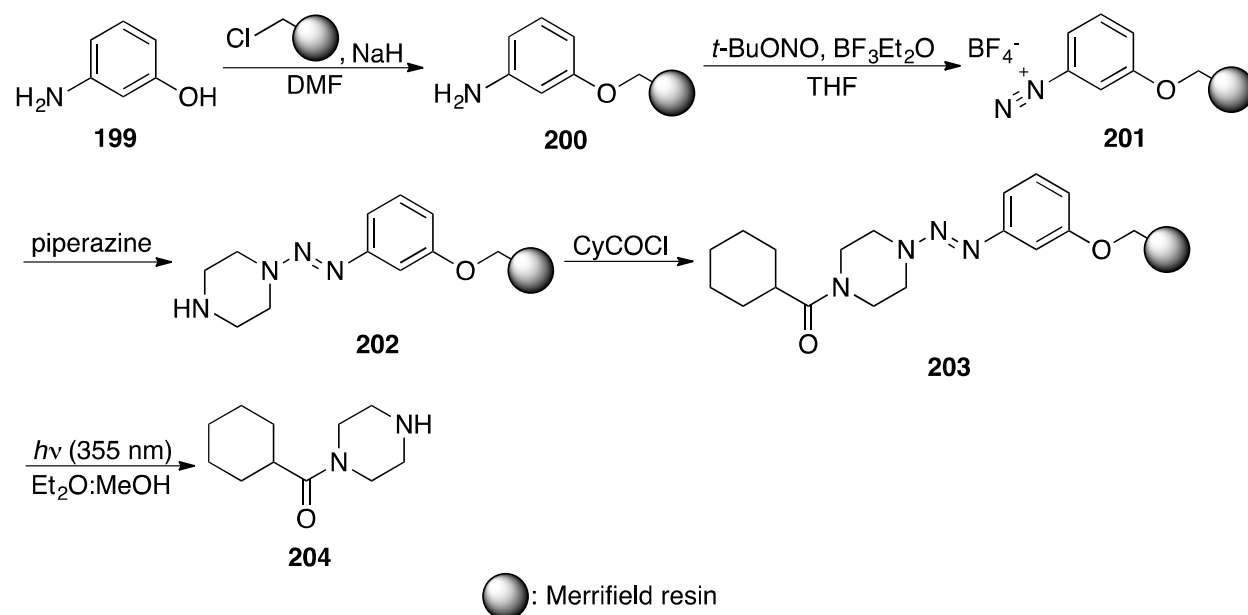
Nicolaou et al. introduced a photolabile linker exploiting the photochemical properties of the 5-bromo-7-nitroindoline group.[84] Photolytic release in the presence of amines provided amides. Intramolecular trapping of an attached amine was also explored in the synthesis of heterocycles. The synthesis started from the indole **187** (Scheme 36).



Scheme 36. A photolabile linker for the synthesis of heterocycles.

Reduction, acylation and methylation led to the methyl ester **189**. Nitration followed by ester hydrolysis and amide formation provided the amide **192**, which after photolytic removal of the acetyl group and ester hydrolysis furnished the linker **194**. Attachment to the solid support and further synthetic elaboration led to the construct **197**, which upon photolytic cyclorelease provided the heterocycle **198** in a yield of 50%.

A triazene-based photolabile linker was developed by Enders et al.[85] 3-Hydroxyaniline (**199**) was attached to a Merrifield resin and diazotation followed by reaction with a secondary amine, such as piperazine, led to the triazene construct **202** (Scheme 37).



Scheme 37. Synthesis and use of triazene-based photolabile linker developed by Enders.

Acylation and photolytic release provided the tertiary amide **204**. Generally, moderate yields and excellent purities were achieved.

Perspectives

As the interest in synthesizing complex molecules, which may be either base- or acid-sensitive, solid support continues to grow, effective protocols for cleavage under mild, neutral conditions remain extremely appealing for SPOS. Fortunately, photolabile linkers provide unique

opportunities for the release of target molecules under mild, neutral conditions by using UV light. Photochemical cleavage generally proceeds with high functional group selectivity, low amounts of side reactions and involve no post-cleavage purification to separate products from reagents. Furthermore, light-cleavable linkers are stable to most standard chemical conditions used in synthetic transformations and consequently, there is no premature cleavage of the library products from the resin beads.

Also, with the development of high-throughput screening techniques, including resin-based bioactivity tests and synthesis of arrays of small molecules on microchips, the importance of photolabile linkers has grown beyond its original purpose. Cleavage can often be performed in aqueous solution, and the liberated products can then be directly employed in biological assays.

The linker of interest may very much depend on the specific application, e.g. some (bio)molecules are not compatible with certain wavelengths and/or specific byproducts. The table below summarizes current photo-cleavable linkers, and the corresponding cleavage conditions. This should prove important for choosing the optimal strategy for a specific application.

Linker	Cleavage Conditions	Product	References
α-Nitrobenzyl linkers			
	$h\nu, \lambda = 350 \text{ nm}$		[10]
	$h\nu, \lambda = 350 \text{ nm}$	X = O: Peptides X = O: Nucleotides X = N: Amido peptides X = O: Small molecules X = O: Saccharides	[11-13, 24],[35] [20-22] [32-34,36] [19, 37] [25-28]
α-nitrobenzyl linkers			
	$h\nu, \lambda = 350 \text{ nm}$	Amido peptides Glycosaminoglycans	[38-40]
	$h\nu, \lambda = 350 \text{ nm}$	Peptides	[41] [42]
α-Nitroveratryl linkers			
	$h\nu, \lambda = 350 \text{ nm}$	Nucleotides Saccharides	[49],[51-52] [48]
	$h\nu, \lambda = 365 \text{ nm}$	Amido peptides Sulfonamidopeptides	[50] [59]
	$h\nu, \lambda = 365 \text{ nm}$		[60]
	$h\nu, \lambda = 365 \text{ nm}$		[62]
	$h\nu, \lambda = 365 \text{ nm}$		[63]
	$h\nu, \lambda = 365 \text{ nm}$		[64-65]

Linker	Cleavage Conditions	Product	References
Phenacyl linkers			
	$h\nu, \lambda = 300\text{-}350\text{ nm}$	Peptides	[67-69]
	$h\nu, \lambda = 350\text{ nm}$	Peptides	[70-71]
Benzoin linkers			
	$h\nu, \lambda = 350\text{ nm}$	Unnatural amino acids	[77]
Pivaloyl linkers			
	$h\nu, \lambda = 280\text{-}340\text{ nm}$	Peptides Small molecules	[80,82] [81]
Other photolabile linkers			
	$h\nu, \lambda = 290\text{ nm}, R^1R^2NH$		[84]
	$h\nu, \lambda = 350\text{ nm}, Bu_3SnH, THF$		[83]
	$h\nu, \lambda = 355\text{ nm}$		[85]

Table 1. Summary of current photocleavable linker strategies for solid-phase syntheses.

Conclusions

More than 40 years have passed since the first publication of a photolabile linker. A wide range of photolabile linkers has since been developed to provide an extensive toolkit for the synthesis of peptides, oligosaccharides, oligonucleotides and small molecules. Photolabile linkers provide a mode of cleavage, which is orthogonal to reaction conditions normally applied with traditional linkers. Furthermore, these linkers are normally cleaved under very mild conditions, which make them attractive for use to release material directly in buffers used for biological studies,[86] and for synthesis circumventing excessive amounts of acidic cleavage reagents. It is expected that the field will expand alongside emerging lighting technologies, such as LED-based systems, which are now being recognized as unique enablers of photochemical organic reactions.

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ABBREVIATIONS

Acm, acetamido; ANP, 3-amino-3-(2-nitrophenyl)propionyl; BME, β -mercaptoethanol; Bn, benzyl; Boc, *t*-butyloxycarbonyl; BOP, benzotriazol-1-yl-oxy-tris(dimethylamino)phosphonium

hexafluorophosphate; BrZ, 2-bromobenzyloxycarbonyl; Bzl, benzyl; ClZ, 2-chlorobenzyloxycarbonyl; DCC, N,N'-dicyclohexylcarbodiimide; DCE, dichloroethane; DDQ, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone; DIC, N,N'-diisopropylcarbodiimide; DIEA, N,N-diisopropylethylamine; DKP, diketopiperazine; DMAP, 4-dimethylaminopyridine; DMB, 3',5'-dimethoxybenzoin; DMF, dimethylformamide; DMP, 2,5-dimethylphenacyl; DMTr, 4,4'-dimethoxytrityl; Fmoc, (9-fluorenylmethyl) carbamate; HATU, Hexafluorophosphate Azabenzotriazole Tetramethyl Uronium; HOBt, 1-hydroxybenzotriazole; LCAA-CPG, long chain aminoalkyl controlled pore glass; LED, light-emitting diode; LG, leaving group; MBHA, 4-Methylbenzhydrylamine; NBB, (3-nitrobenzamido)benzyl; NBH, 2-nitrobenzhydryl; NBOC, 2-nitrobenzyloxycarbonyl; NBS, N-bromosuccinimide; NMM, N-methylmorpholine; NMP, N-methylpyrrolidone; NVOC, 6-nitroveratryloxycarbonyl; PEG, polyethylene glycol; PPOA, [4-(2-bromopropionyl)phenoxy]acetic acid; PS, polystyrene; SPPS, solid-phase peptide synthesis; SPOS, solid-phase organic synthesis; TBDPS, tetrabutylphenylsilyl; TBTU, O-(benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate; TFA, trifluoroacetic acid; TFP, tri-2-Furylphosphine; THF, tetrahydrofuran; Thy, thymine; TCA, trichloroacetic acid; TLC, thin layer chromatography; Tos, p-toluenesulfonyl; UNP-Gal, uridine diphosphate galactose; Z, carbobenzyloxy (benzyloxycarbonyl).

REFERENCES

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- [1]: Merrifield, R. B. Solid Phase Peptide Synthesis. I. The Synthesis of a Tetrapeptide. *J. Am. Chem. Soc.* **1963**, 85, 2149–2154.

[2]: Testero, S. A.; Mata, E. G. *J. Prospect of Metal-Catalyzed C–C Forming Cross-Coupling Reactions in Modern Solid-Phase Organic Synthesis. Comb. Chem.* **2008**, *10*, 487–497.

[3]: Hermkens, P. H. H.; Ottenheijm, H. C. J.; Rees, D. Solid-phase organic reactions: A review of the recent literature. *Tetrahedron* **1996**, *52*, 4527–4554.

[4]: Hermkens, P. H. H.; Ottenheijm, H. C. J.; Rees, D. C. Solid-phase organic reactions II: A review of the literature Nov 95–Nov 96. *Tetrahedron* **1997**, *53*, 5643–5678.

[5]: Booth, S.; Hermkens, P. H. H.; Ottenheijm, H. C. J.; Rees, D. C. Solid-phase organic reactions III: A review of the literature Nov 96–Dec 97. *Tetrahedron* **1998**, *54*, 15385–15443.

[6]: Hall, S. E. Recent advances in solid phase synthesis. *Mol. Divers.* **1999**, *4*, 131–142.

[7]: Dolle, R. E.; Nelson, K. H., Jr. Comprehensive Survey of Combinatorial Library Synthesis: 1998. *J. Comb. Chem.* **1999**, *1*, 235–282.

[8]: Taylor, S. J.; Morken, J. P. Thermographic Selection of Effective Catalysts from an Encoded Polymer-Bound Library. *Science* **1998**, *280*, 267–270.

[9]: Bamford, C. H.; Norrish, R. G. W. Primary Photochemical Reactions. Part VII. Photochemical Decomposition of Isovaleraldehyde and Di-n-propyl Ketone. *J. Chem. Soc.* **1935**, 1504-1511.

[10]: Rich, D. H.; Gurwara, S. K. *J. Removal of protected peptides from an ortho-nitrobenzyl resin by photolysis. Chem. Soc., Chem. Commun.* **1973**, 610–611.

[11]: Rich, D. H.; Gurwara, S. K. Preparation of a new o-nitrobenzyl resin for solid-phase synthesis of tert-butyloxycarbonyl-protected peptide acids. *J. Am. Chem. Soc.* **1975**, *97*, 1575–1579.

[12]: Tam, J. P.; Tjoeng, F. S.; Merrifield, R. B. Design and synthesis of multidetachable resin supports for solid-phase peptide synthesis. *J. Am. Chem. Soc.* **1980**, *102*, 6117–6127.

[13]: Voss, C; Dimarchi, R; Whitney, D. B. ; Tjoeng, F. S.; Merrifield, R. B.; Tam, J. P. Synthesis of the protected tridecapeptide (56-68) of the VH domain of mouse myeloma immunoglobulin M603 and its reattachment to resin supports. *Int. J. Pept. Protein Res.* **1983**, *22*, 204–213.

[14]: Barany, G.; Albericio, F. Three-dimensional orthogonal protection scheme for solid-phase peptide synthesis under mild conditions. *J. Am. Chem. Soc.* **1985**, *107*, 4936–4942.

[15]: Giralt, E.; Albericio, F.; Pedroso, E.; Granier, C.; Van Rietschoten, J. Convergent solid phase peptide synthesis. II. Synthesis of the 1–6 apamin protected segment on a NBB-resin. Synthesis of apamin. *Tetrahedron* **1982**, *38*, 1193–1201.

[16]: Giralt, E.; Eritja, R.; Pedroso, E. Convergent solid phase peptide synthesis-III: Synthesis of the 44-52 protected segment of the toxin II of androctonus australis hector. *Tetrahedron* **1986**, *42*, 691–698.

[17]: Lloyd-Williams, P.; Gairi, M.; Albericio, F.; Giralt, E. Convergent solid-phase peptide synthesis. X. Synthesis and purification of protected peptide fragments using the photolabile Nbb-resin. *Tetrahedron* **1991**, *47*, 9867–9880.

[18]: Pillai, V. N. R.; Renil, M.; Haridasan, V. K. Synthesis of thioredoxin partial sequences on a polyethyleneglycol-grafted polystyrene support with a photolytically detachable 2-nitrobenzyl anchoring group. *Indian J. Chem., Sect. B* **1991**, *30B*, 205–212.

[19]: Baldwin, J. J.; Burbaum, J. J.; Henderson, I.; Ohlmeyer, M. H. J. Synthesis of a Small Molecule Combinatorial Library Encoded with Molecular Tags. *J. Am. Chem. Soc.* **1995**, *117*, 5588–5589.

[20]: Greenberg, M. M. Photochemical cleavage of oligonucleotides from solid phase supports. *Tetrahedron Lett.* **1993**, *34*, 251–254.

[21]: Matray, T. J.; Greenberg, M. M. Site-Specific Incorporation of the Alkaline Labile, Oxidative Stress Product (5R)-5,6-Dihydro-5-hydroxythymidine in an Oligonucleotide. *J. Am. Chem. Soc.* **1994**, *116*, 6931–6932.

[22]: Greenberg, M. M.; Gilmore, J. L. Cleavage of Oligonucleotides from Solid-Phase Supports Using o-Nitrobenzyl Photochemistry. *J. Org. Chem.* **1994**, *59*, 746–753.

[23]: Alsina, J.; Chiva, C.; Ortiz, M.; Francesc, R.; Giralt, E.; Albericio, F. Active carbonate resins for solid-phase synthesis through the anchoring of a hydroxyl function. Synthesis of cyclic and alcohol peptides. *Tetrahedron Lett.* **1997**, *38*, 883–886.

[24]: Tam, J. P.; Dimarchi, R. D.; Merrifield, R. B. Photolabile multi-detachable *p*-alkoxybenzyl alcohol resin supports for peptide fragment or semi-synthesis. *Int. J. Pept. Protein Res.* **1980**, *16*, 412–425.

[25]: Zehavi, U.; Sadeh, S.; Herchman, M. Enzymic synthesis of oligosaccharides on a polymer support light-sensitive, substituted polyacrylamide beads. *Carbohydr. Res.* **1983**, *124*, 23–34.

[26]: Nicolaou, K. C.; Winssinger, N.; Pastor, J.; DeRoose, F. A General and Highly Efficient Solid Phase Synthesis of Oligosaccharides. Total Synthesis of a Heptasaccharide Phytoalexin Elicitor (HPE). *J. Am. Chem. Soc.* **1997**, *119*, 449–450.

[27]: Nicolaou, K. C.; Watanabe, N.; Li, J.; Pastor, J.; Winssinger, N. Solid-phase synthesis of oligosaccharides: construction of a dodecasaccharide. *Angew. Chem. Int. Ed.* **1998**, *37*, 1559–1561.

[28]: Kantchev, E. A. B.; Parquette, J. R. Hyper-branched polyester supported disaccharide synthesis: The effect of loading level on glycosylation efficiency *Synlett* **2005**, 1567–1570.

[29]: Armstrong, R. W.; Combs, A. P.; Tempest, P. A.; Brown, S. D.; Keating, T. A. Multiple-Component Condensation Strategies for Combinatorial Library Synthesis. *Acc. Chem. Res.* **1996**, *29*, 123–131.

[30]: Johnsson, R.; Lackey, J. G.; Bogojeski, J. J.; Damha, M. J. New light labile linker for solid phase synthesis of 2'-O-acetal-ester oligonucleotides and applications to siRNA prodrug development. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 3721–3725.

[31]: Hasan, A.; Stengele, K.-P.; Giegrich, H.; Cornwell, P.; Isham, K. R.; Sachleben, R. A.; Pfeleiderer, W.; Foote, R. S. Photolabile protecting groups for nucleosides: synthesis and photodeprotection rates. *Tetrahedron* **1997**, *53*, 4247–4264.

[32]: Rich, D. H.; Gurwara, S. K. Removal of C-terminal peptide amides from a 3-nitro-4-aminomethyl-benzoyl amide resin by photolysis. *Tetrahedron Lett.* **1975**, *16*, 301–304.

[33]: Hammer, R. P.; Albericio, F.; Gera, L.; Barany, G. Practical approach to solid-phase synthesis of C-terminal peptide amides under mild conditions based on a photolysable anchoring linkage. *Int. J. Pept. Protein Res.* **1990**, *36*, 31–45.

[34]: Ajayaghosh, A.; Pillai, V. N. R. Solid-phase synthesis of N-methyl- and N-ethylamides of peptides using photolytically detachable [(3-nitro-4-[(alkylamino)methyl]benzamido)methyl]polystyrene resin. *J. Org. Chem.* **1990**, *55*, 2826–2829.

[35]: Renil, M.; Pillai, V. N. R. Synthesis of fully protected peptides on a tetraethyleneglycol diacrylate (TTEGDA)-crosslinked polystyrene support with a photolytically detachable 2-nitrobenzyl anchoring group. *Tetrahedron Lett.* **1994**, *35*, 3809–3812.

[36]: Kumar, K. S.; Pillai, V. N. R. Synthesis of peptide-N-alkylamides on a new PS-TTEGDA polymer support using photolabile anchoring group. *Tetrahedron* **1999**, *55*, 10437–10446.

[37]: Hintersteiner, M.; Ambrus, G.; Bednenko, J.; Schmied, M.; Knox, A. J. S.; Meisner, N.-C.; Gstach, H.; Seifert, J.-M.; Singer, E. L.; Gerace, L.; Auer, M. Identification of a Small Molecule Inhibitor of Importin β Mediated Nuclear Import by Confocal On-Bead Screening of Tagged One-Bead One-Compound Libraries. *ACS Chem. Biol.* **2010**, *5*, 967–979.

[38]: Eller, S.; Collot, M.; Yin, J.; Hahm, H. S.; Seeberger, P. H. Automated Solid-Phase Synthesis of Chondroitin Sulfate Glycosaminoglycans. *Angew. Chem. Int. Ed.* **2013**, *52*, 5858–5861.

[39]: Calin, O; Eller, S.; Seeberger, P. H. Automated Polysaccharide Synthesis: Assembly of a 30mer Mannoside. *Angew. Chem. Int. Ed.* **2013**, *52*, 5862–5865.

[40]: Weishaupt, M. W.; Matthies, S.; Seeberger, P. H. Automated Solid-Phase Synthesis of a β -(1,3)-Glucan Dodecasaccharide. *Chem. Eur. J.* **2013**, *19*, 12497–12503.

[41]: Ajayaghosh, A; Pillai, V. N. R. Polymer-supported synthesis of protected peptide segments on a photosensitive o-nitro(a-methyl)bromobenzyl resin. *Tetrahedron* **1988**, *44*, 6661–6666.

[42]: Ajayaghosh, A; Pillai, V. N. R. 2'-Nitrobenzhydryl polystyrene resin: a new photosensitive polymeric support for peptide synthesis. *J. Org. Chem.* **1987**, *52*, 5714–5717.

[43]: Ajayaghosh, A; Pillai, V. N. R. Solid-phase synthesis and C-terminal amidation of peptides using a photolabile o-nitrobenzhydrylaminopolystyrene support. *Tetrahedron Lett.* **1995**, *36*, 777–780.

[44]: Brown, B. B.; Wagner, D. S.; Geysen, H. M. A single-bead decode strategy using electrospray ionization mass spectrometry and a new photolabile linker: 3-Amino-3-(2-nitrophenyl)propionic acid. *Mol. Div.* **1995**, *1*, 4–12

[45]: Sternson, S. M.; Schreiber, S. L. An acid- and base-stable o-nitrobenzyl photolabile linker for solid phase organic synthesis. *Tetrahedron Lett.* **1998**, *39*, 7451–7454.

[46]: Rodebaugh, R.; Fraser-Reid, B.; Geysen, H. M. A new o-nitrobenzyl photocleavable linker for solid phase synthesis. *Tetrahedron Lett.* **1997**, *97*, 7653–7656.

[47]: Ryba, T. D.; Harran, P. G. Two Useful Photolabile Surfaces for Solid-Phase Synthesis. *Org. Lett.* **2000**, *2*, 851–853.

[48]: Zehavi, U.; Patchornik, A. Oligosaccharide synthesis on a light-sensitive solid support. I. Polymer and synthesis of isomaltose (6-O-.alpha.-D-glucopyranosyl-D-glucose). *J. Am. Chem. Soc.* **1973**, *95*, 5673–5677.

[49]: Yoo, D. J.; Greenberg, M. M. Synthesis of Oligonucleotides Containing 3'-Alkyl Carboxylic Acids Using Universal, Photolabile Solid Phase Synthesis Supports. *J. Org. Chem.* **1995**, *60*, 3358–3364.

[50]: Holmes, C. P.; Jones, D. G. Reagents for Combinatorial Organic Synthesis: Development of a New *o*-Nitrobenzyl Photolabile Linker for Solid Phase Synthesis. *J. Org. Chem.* **1995**, *60*, 2318–2319.

[51]: Venkatesan, H.; Greenberg, M. M. Improved Utility of Photolabile Solid Phase Synthesis Supports for the Synthesis of Oligonucleotides Containing 3'-Hydroxyl Termini. *J. Org. Chem.* **1996**, *61*, 525–529.

[52]: Poijaervi, P.; Heinonen, P.; Virta, P.; Loennberg, H. 2,2-Bis(ethoxycarbonyl)- and 2-(Alkylaminocarbonyl)-2-cyano-Substituted 3-(Pivaloyloxy)propyl Groups as Biodegradable Phosphate Protections of Oligonucleotides. *Bioconjugate Chem.* **2005**, *16*, 1564–1571.

[53]: Holmes, C. P. Model Studies for New *o*-Nitrobenzyl Photolabile Linkers: Substituent Effects on the Rates of Photochemical Cleavage. *J. Org. Chem.* **1997**, *62*, 2370–2380.

[54]: Teague, S. J. Facile synthesis of a o-nitrobenzyl photolabile linker for combinatorial chemistry. *Tetrahedron Lett.* **1996**, *37*, 5751–5754.

[55]: Whitehouse, D. L.; Savinov, S. N.; Austin, D. J. An improved synthesis and selective coupling of a hydroxy based photolabile linker for solid phase organic synthesis. *Tetrahedron Lett.* **1997**, *38*, 7851–7852.

[56]: McKeown, S. C.; Watson, S. P.; Carr, R. A. E.; Marshall, P. A photolabile carbamate based dual linker analytical construct for facile monitoring of solid phase chemistry: ‘TLC’ for solid phase? *Tetrahedron Lett.* **1999**, *40*, 2407–2410.

[57]: Gea, A.; Farcy, N.; Roqué I Rossell, N.; Martins, J. C.; De Clercq, P. J.; Madder, A. Solid-Supported Synthesis of Highly Functionalized Tripodal Peptides with Flexible but Preorganized Geometry: Towards Potential Serine Protease Mimics. *Eur. J. Org. Chem.* **2006**, 4135–4146.

[58]: Lin, Q.; Blackwell, H. E. Rapid synthesis of diketopiperazine macroarrays via Ugi four-component reactions on planar solid supports. *Chem. Commun.* **2006**, 2884–2886.

[59]: Gennari, C.; Longari, C.; Ressel, S.; Salom, B.; Piarulli, U.; Ceccarelli, S.; Mielgo, A. Synthesis of Combinatorial Libraries of Vinylogous Sulfonamidopeptides (vs-Peptides). *Eur. J. Org. Chem.* **1998**, 2437–2449.

[60]: Ruhland, B.; Bhandari, A.; Gordon, E. M.; Gallop, M. A. Solid-Supported Combinatorial Synthesis of Structurally Diverse β -Lactams. *J. Am. Chem. Soc.* **1996**, *118*, 253–254.

[61]: Minkwitz, R.; Meldal, M. Application of a Photolabile Backbone Amide Linker for Cleavage of Internal Amides in the Synthesis towards Melanocortin Subtype-4 Agonists. *QSAR Comb. Sci.* **2005**, *24*, 343–353.

[62]: Qvortrup, K; Nielsen, T. E. A photolabile linker for the solid-phase synthesis of 4-substituted NH-1,2,3-triazoles. *Chem. Commun.* **2011**, *47*, 3278–3280.

[63]: Qvortrup, K; Komnatnyy, V. V.; Nielsen, T. E. A Photolabile Linker for the Solid-Phase Synthesis of Peptide Hydrazides and Heterocycles. *Org. Lett.* **2014**, *16*, 4782–4785.

[64]: Qvortrup, K; Nielsen, T. E. In-Bead Screening of Hydroxamic Acids for the Identification of HDAC Inhibitors. *Angew. Chem. Int. Ed. Engl.* **2016**, *14*, 4472–4475.

[65]: Qvortrup, K.; Petersen, R. G.; Dohn, A. O.; Møller, K. B.; Nielsen, T. E. Solvent-Controlled Chemoselectivity in the Photolytic Release of Hydroxamic Acids and Carboxamides from Solid Support. *Org. Lett.* **2017**, *12*, 3263–3266.

[66]: Kim, J.; Kyeong, S.; Shin, D.-S.; Yeo, S.; Yim, J.; Lee, Y.-S. Facile Synthesis of N-(9-Fluorenylmethyloxycarbonyl)-3-amino-3-(4,5-dimethoxy-2-nitrophenyl)propionic Acid as a Photocleavable Linker for Solid-Phase Peptide Synthesis. *Synlett* **2013**, 733–736.

[67]: Sheehan, J.C.; Umezawa, K. Phenacyl photosensitive blocking groups, *J. Org. Chem.* **1973**, *38*, 3771-3774.

[68]: Wang, S.-S.; Solid phase synthesis of protected peptides via photolytic cleavage of the .alpha.-methylphenacyl ester anchoring linkage. *J. Org. Chem.* **1976**, *41*, 3258–3261.

[69]: Tjoeng, F. S.; Heavner, G. A. Synthesis of a new photolabile support. 4-(2-Chloropropionyl)phenylacetamidomethyl-resin and its application in solid-phase peptide synthesis. *J. Org. Chem.* **1983**, *48*, 355–359.

[70]: Bellof, D.; Mutter, M. A new phenacyl-type handle for polymer supported peptide synthesis. *Chimia* **1985**, *39*, 317–320.

[71]: Abraham, N. A.; Fazal, G.; Ferland, J. M.; Rakhit, S.; Gauthier, J. A new solid-phase strategy for the synthesis of mammalian glucagon. *Tetrahedron Lett.* **1991**, *32*, 577–580.

[72]: Flickinger, S. T.; Patel, M.; Binkowski, B. F.; Lowe, A. M.; Li, M.-H.; Kim, C.; Cerrina, F.; Belshaw, P. J. Spatial Photorelease of Oligonucleotides, Using a Safety-Catch Photolabile Linker. *Org. Lett.* **2006**, *8*, 2357–2360.

[73]: Sucholeiki, I. Solid-phase photochemical C-S bond cleavage of thioethers-a new approach to the solid-phase production of non-peptide molecules. *Tetrahedron Lett.* **1994**, *35*, 7307–7310.

[74]: Forman, F. W.; Sucholeiki, I. Solid-Phase Synthesis of Biaryls via the Stille Reaction. *J. Org. Chem.* **1995**, *60*, 523–528.

[75]: Rock, R. S.; Chan, S. I. Synthesis and Photolysis Properties of a Photolabile Linker Based on 3'-Methoxybenzoin. *J. Org. Chem.* **1996**, *61*, 1526–1529.

[76]: Routledge, A.; Abell, C.; Balasubramanian, S. The use of a dithiane protected benzoin photolabile safety catch linker for solid-phase synthesis. *Tetrahedron Lett.* **1997**, *38*, 1227–1230.

[77]: Lee, H. B.; Balasubramanian, S. Studies on a Dithiane-Protected Benzoin Photolabile Safety Catch Linker for Solid-Phase Synthesis. *J. Org. Chem.* **1999**, *64*, 3454–3460.

[78]: Cano, M.; Ladlow, M.; Balasubramanian, S. Practical Synthesis of a Dithiane-Protected 3',5'-Dialkoxybenzoin Photolabile Safety-Catch Linker for Solid-Phase Organic Synthesis. *J. Org. Chem.* **2002**, *67*, 129–135

[79]: Chumachenko, N.; Novikov, Y.; Shoemaker, R. K.; Copley, S. D. A Dimethyl Ketal-Protected Benzoin-Based Linker Suitable for Photolytic Release of Unprotected Peptides. *J. Org. Chem.* **2011**, *76*, 9409–9416.

[80]: Peukert, S.; Giese, B. The Pivaloylglycol Anchor Group: A New Platform for a Photolabile Linker in Solid-Phase Synthesis. *J. Org. Chem.* **1998**, *63*, 9045–9051.

[81]: Glatthar, R.; Giese, B. A New Photocleavable Linker in Solid-Phase Chemistry for Ether Cleavage. *Org. Lett.* **2000**, *2*, 2315–2317.

[82]: Kessler, M.; Glatthar, R.; Giese, B.; Bochet, C. G. Sequentially Photocleavable Protecting Groups in Solid-Phase Synthesis. *Org. Lett.* **2003**, *5*, 1179–1181.

[83]: Horton, J. R.; Stamp, L. M.; Routledge, A. A photolabile 'traceless' linker for solid-phase organic synthesis. *Tetrahedron Lett.* **2000**, *41*, 9181–9184.

[84]: Nicolaou, K. C.; Safina, B. S.; Winssinger, N. A new photolabile linker for the photoactivation of carboxyl groups. *Synlett* **2001**, 900–903.

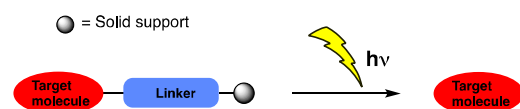
[85]: Enders, D.; Rijksen, C.; Bremus-Köbberling, E.; Gillner, A.; Köbberling, J. A triazene-based new photolabile linker in solid phase chemistry. *Tetrahedron Lett.* **2004**, *45*, 2839–2841.

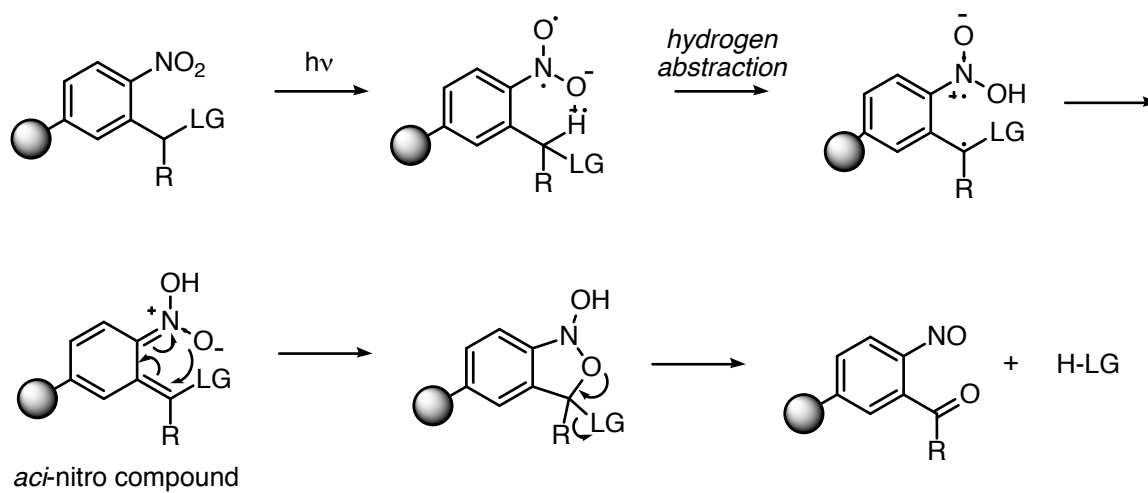
[86]: (a) Fluxà, V. S.; Maillard, N.; Page, M. G. P.; Reymond, J.-L. Bead diffusion assay for discovering antimicrobial cyclic peptides. *Chem. Commun.* **2011**, *47*, 1434–1436. (b) Shin, D.; Kim, Y.-G.; Kim, E.-M.; Kim, M.; Park, H.; Kim, J.-H.; Lee, B.-S.; Kim, B.; Lee, Y. Solid-Phase Peptide Library Synthesis on HiCore Resin for Screening Substrate Specificity of Brk Protein Tyrosine Kinase. *J. Comb. Chem.* **2008**, *10*, 20–23. (c) Guillena, G.; Halkes, K. M.; Rodríguez, G.; Batema, G. D.; van Koten, G.; Kamerling, J. P. Organoplatinum(II) Complexes as a Color Biomarker in Solid-Phase Peptide Chemistry and Screening. *Org. Lett.* **2003**, *5*, 2021–2024. (d) Johannesson, P.; Erdélyi, M.; Lindeberg, G.; Frändberg, P.-A.; Nyberg, F.; Karlén, A.; Hallberg, A. Bicyclic Tripeptide Mimetics with Reverse Turn Inducing Properties. *J. Med. Chem.* **2004**, *47*, 6009–6019. (e) Miyashita, M.; Otake, Y.; Oda, M.; Miyagawa, H. Development of a High-Throughput Screening Method Using a Cell-Based, Lawn Format Assay for the Identification of Novel Plant Defense Activators from Combinatorial Peptide Libraries. *J. Agric. Food Chem.* **2007**, *55*, 806–811. (f) Maillard, N.; Biswas, R.; Darbre, T.; Reymond, J.-L. Combinatorial Discovery of Peptide Dendrimer Enzyme Models Hydrolyzing Isobutyryl Fluorescein. *ACS Comb. Sci.* **2011**, *13*, 310–320. (g) Maillard, N.; Darbre, T.; Reymond, J.-L. Combinatorial discovery of peptide dendrimer enzyme models hydrolyzing isobutyryl fluorescein. *J. Comb. Chem.* **2009**, *11*, 667–675. (h) Tornøe, C. W.; Sanderson, S. J.; Mottram, J. C.; Coombs, G. H.; Meldal, M. Combinatorial Library of Peptidotriazoles: Identification of [1,2,3]-Triazole Inhibitors against a Recombinant *Leishmania mexicana* Cysteine Protease. *J. Comb. Chem.* **2004**, *6*, 312–324. (i) Usui, K.; Kikuchi, T.; Tomizaki, K.; Kakiyama, T.; Mihara, H. A novel array format for monitoring cellular uptake using a photo-cleavable linker for peptide release. *Chem. Commun.* **2013**, *49*, 6394–6396. (j) Zhou, G.; Khan, F.; Dai, Q.; Sylvester, J. E.; Kron, S. J. Photocleavable peptide–oligonucleotide conjugates for protein kinase

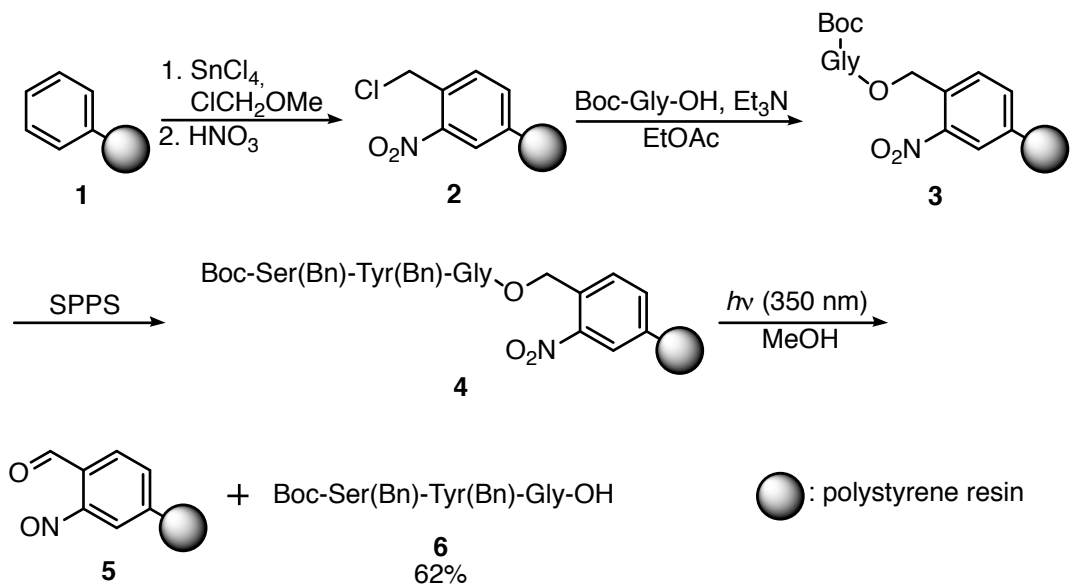
assays by MALDI-TOF MS. *Mol. BioSyst.* **2012**, 8, 2395–2404. (k) Bodé, C. A.; Bechet, T.; Prodhomme, E.; Gheysen, K.; Gregoir, P.; Martins, J. C.; Muller, C. P.; Madder, A. Towards the conformational mimicry of the measles virus HNE loop: design, synthesis and biological evaluation of a cyclic bile acid–peptide conjugate. *Org. Biomol. Chem.* **2009**, 7, 3391–3399. (l) Verzele, D.; Madder, A. Synthetic Progress in cMyc-Max Oncoprotein Miniaturization: Semi-Online Monitoring Gives Solid-Phase Access to Hydrophobic b(-HLH-)ZIP Peptidosteroid Tweezers. *Eur. J. Org. Chem.* **2013**, 673–687. (m) Boeijen, A.; Liskamp, R. M. J. Solid-Phase Synthesis of Oligourea Peptidomimetics. *Eur. J. Org. Chem.* **1999**, 2127–2135. (n) Jang, K.; Sato, K.; Mawatari, K.; Konno, T.; Ishihara, K.; Kitamori, T. Surface modification by 2-methacryloyloxyethyl phosphorylcholine coupled to a photolabile linker for cell micropatterning. *Biomaterials* **2009**, 30, 1413–1420.

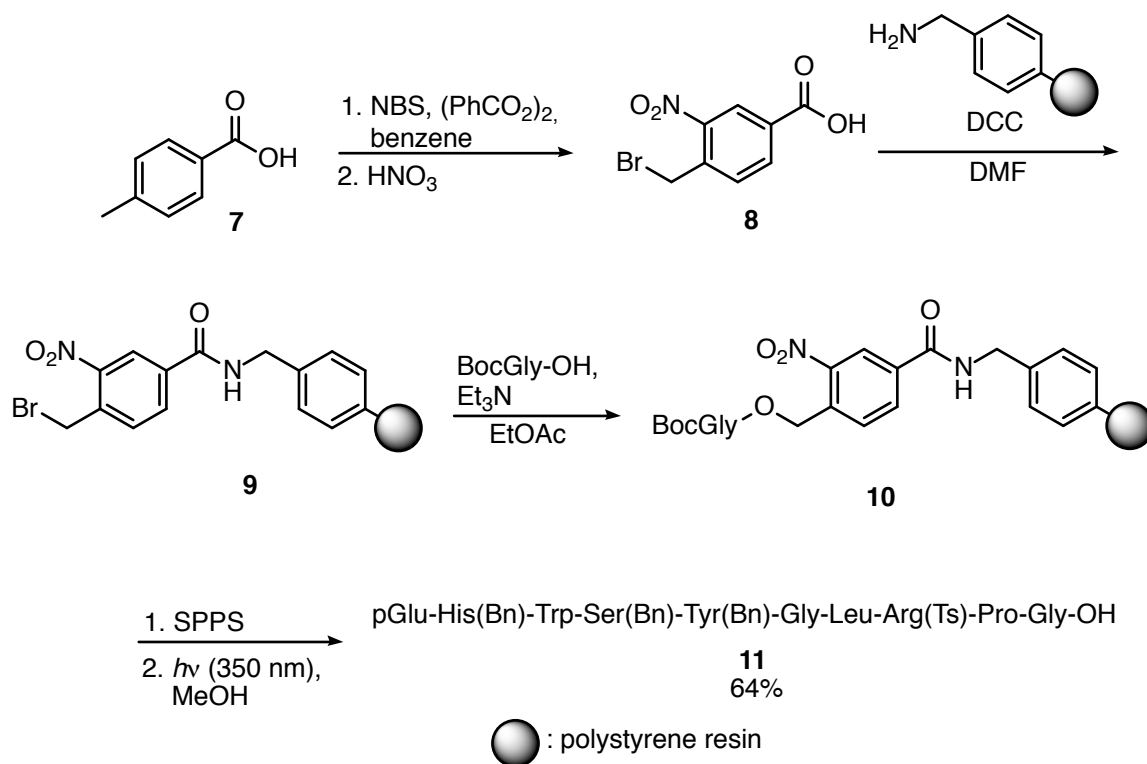
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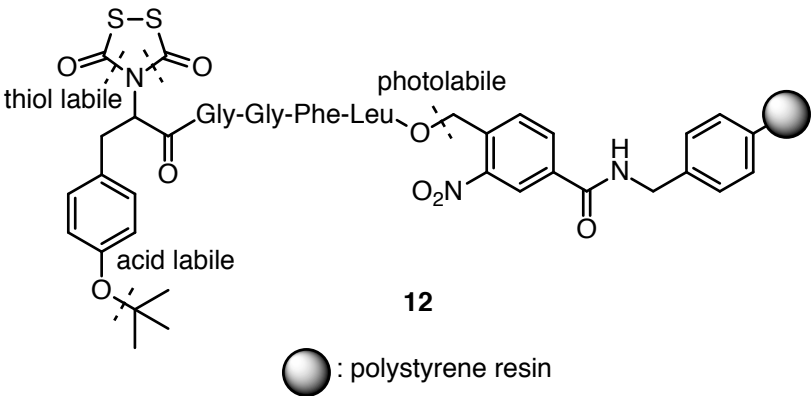
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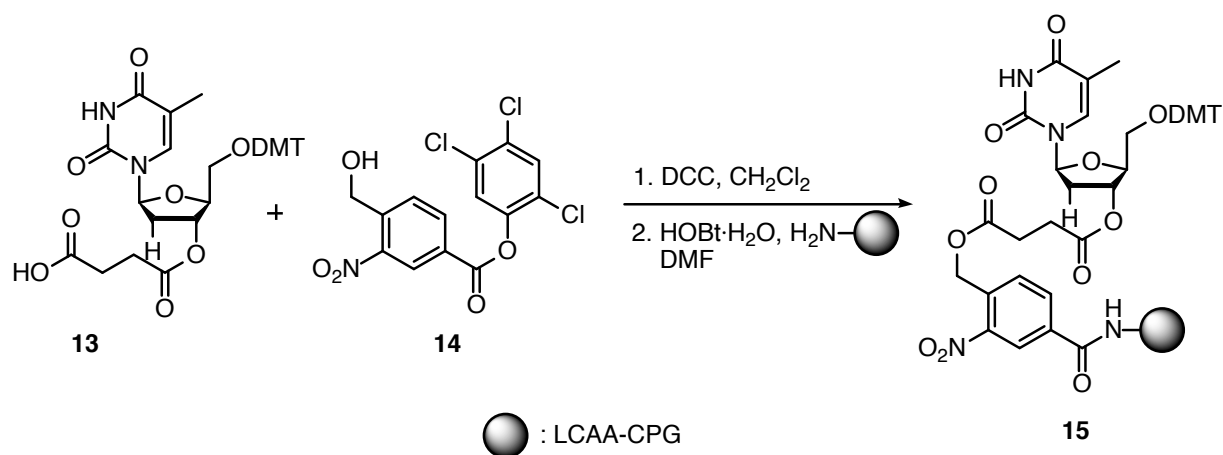


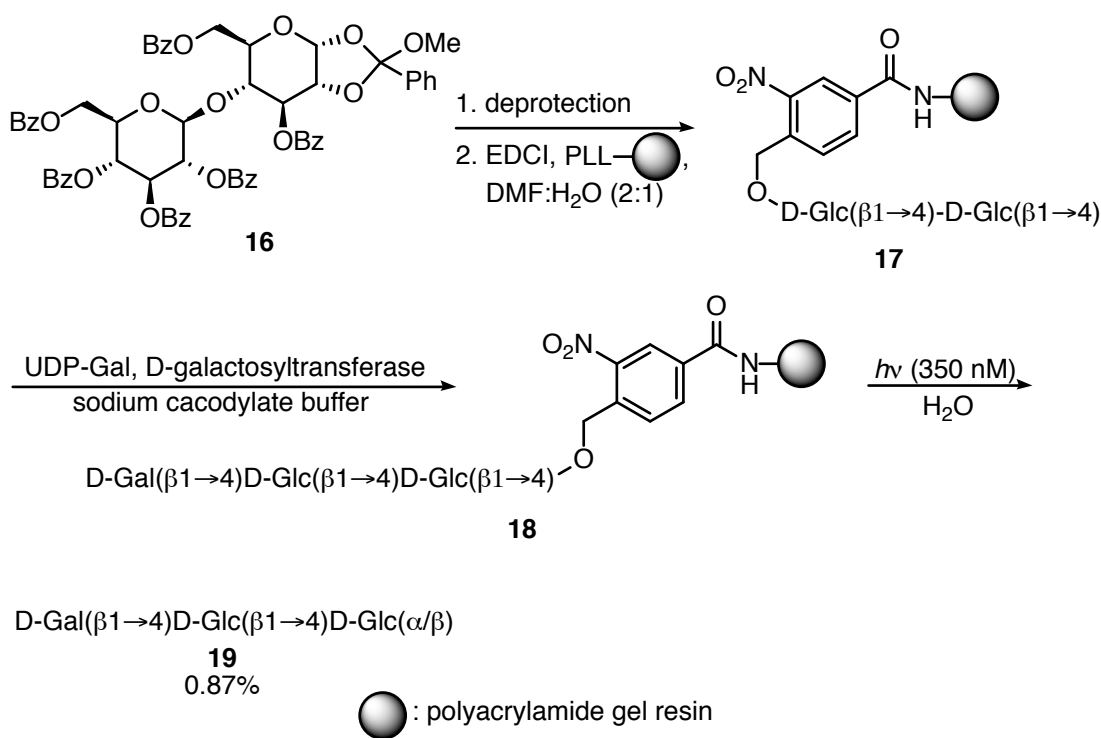


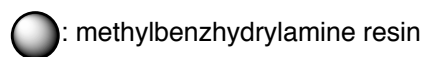


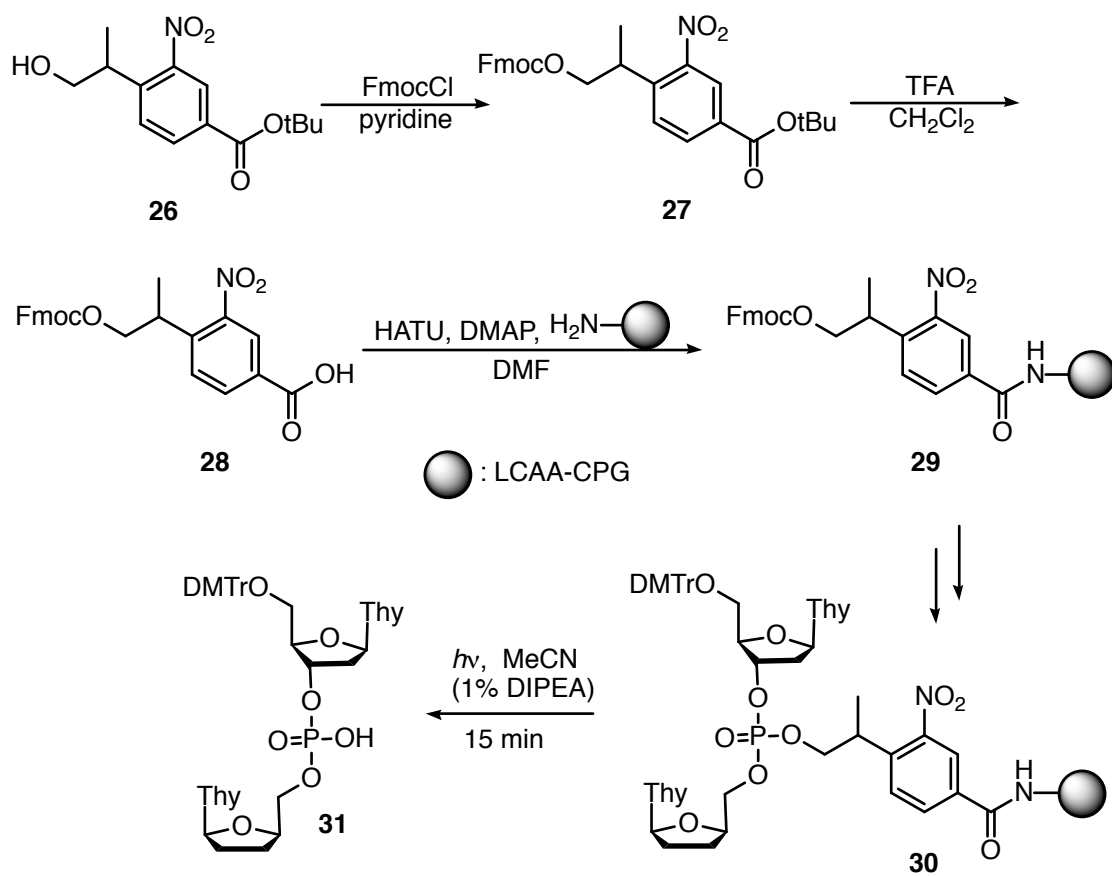


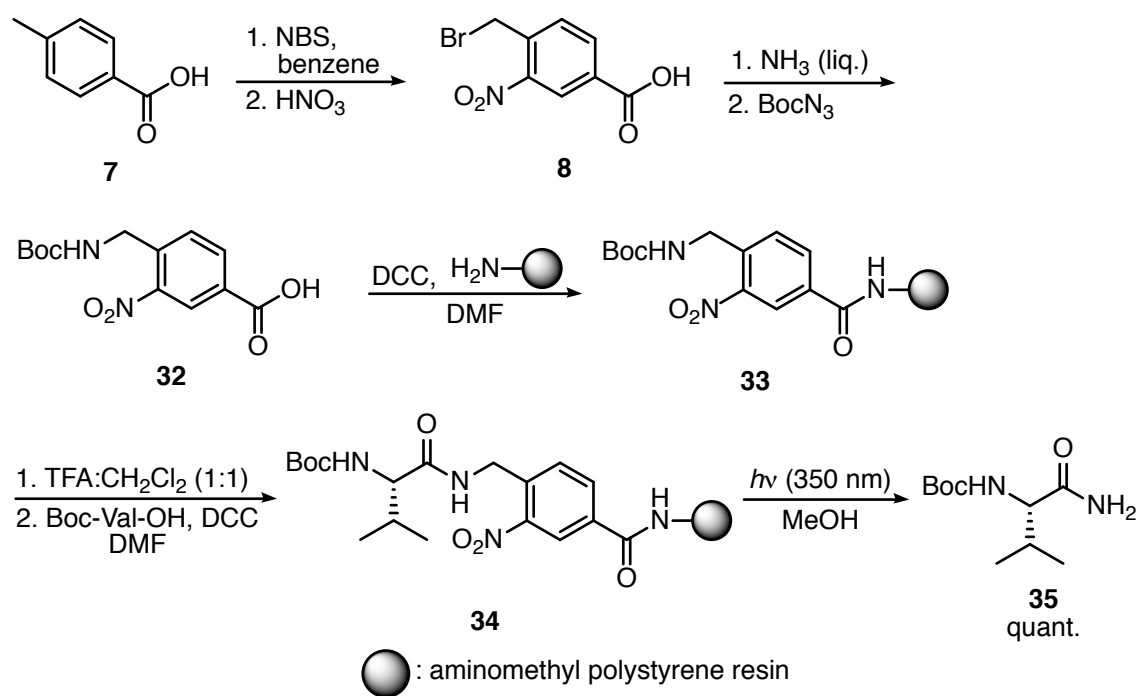


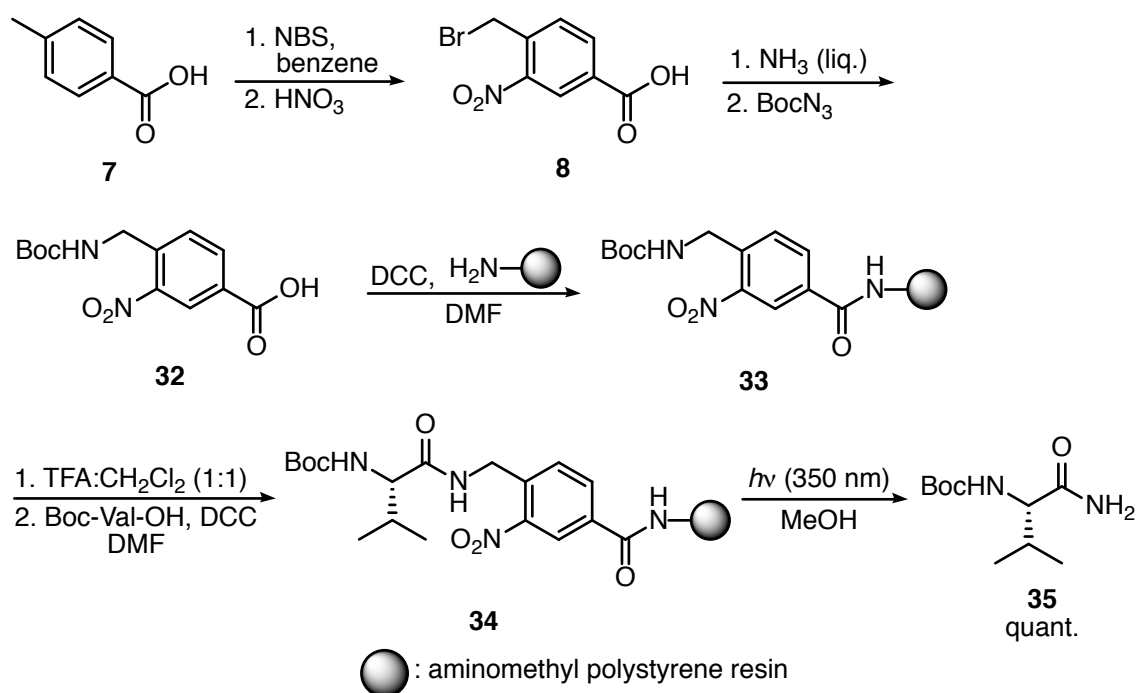


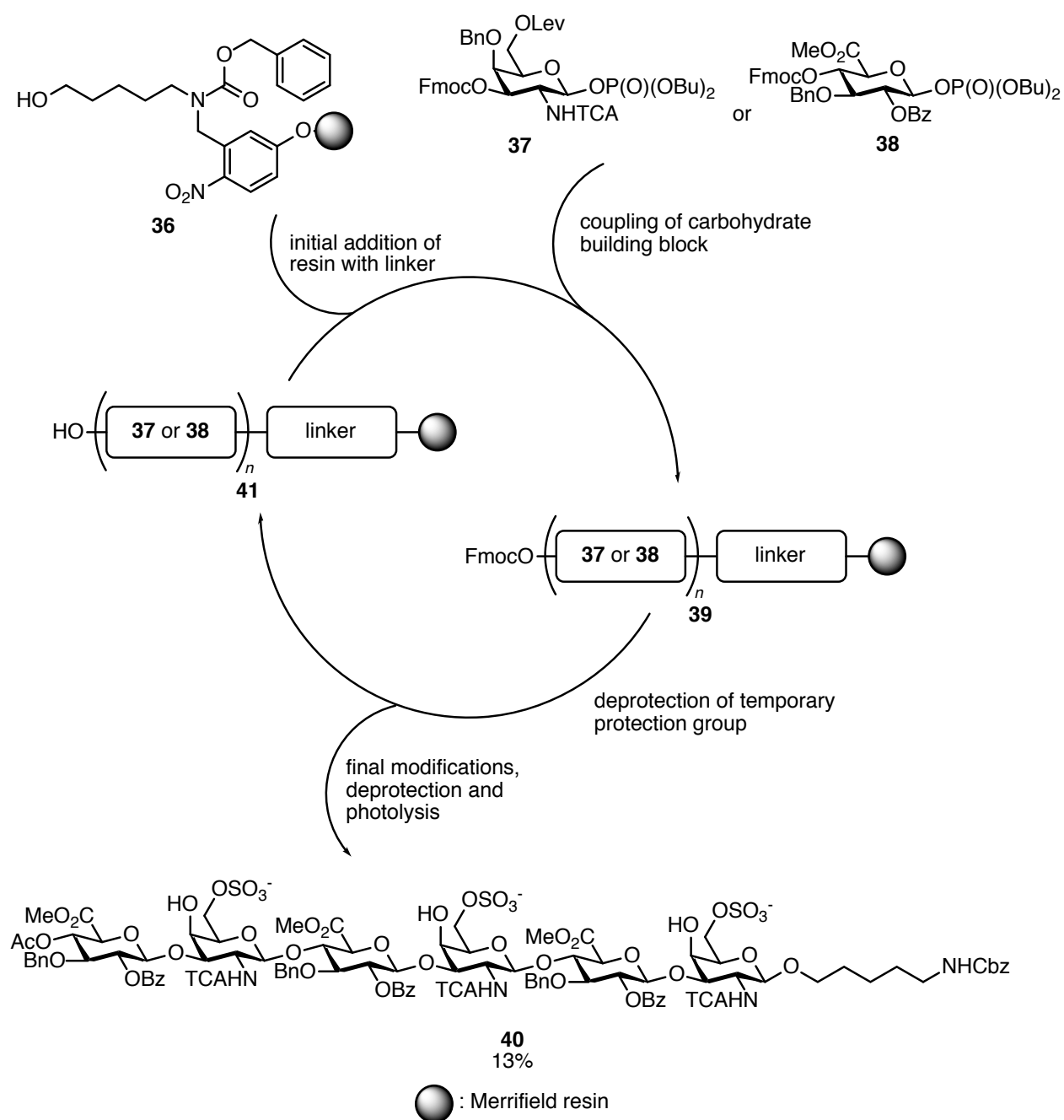


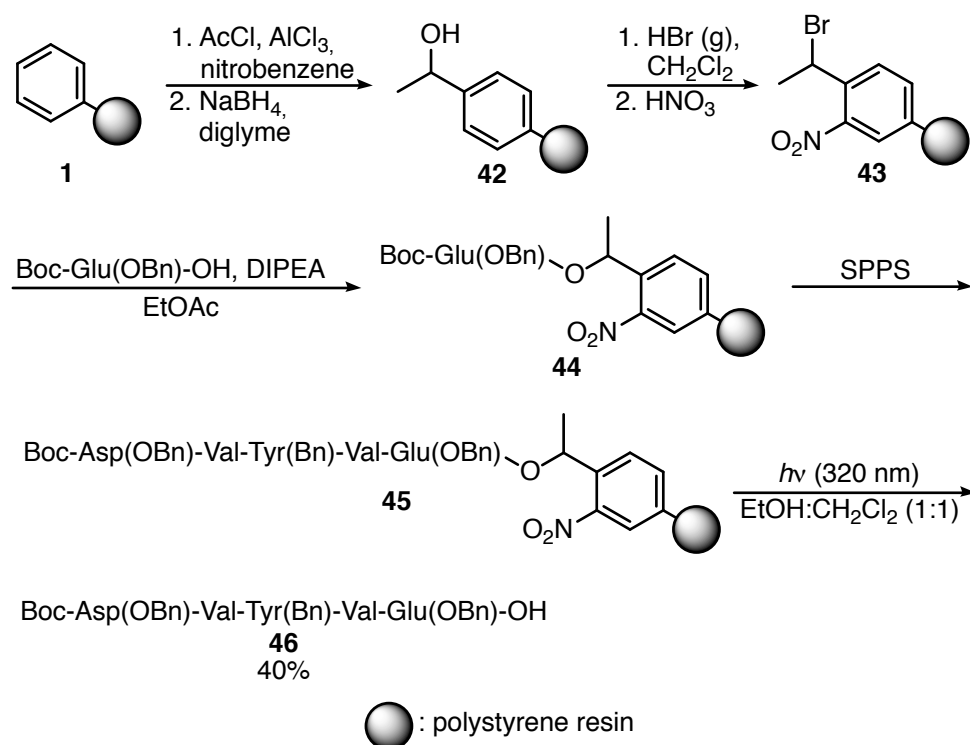


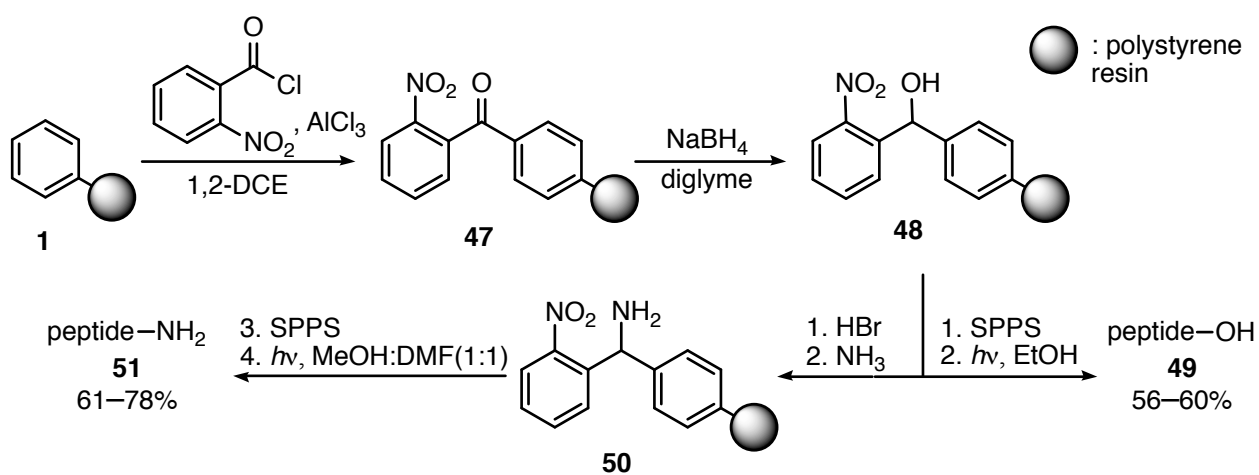


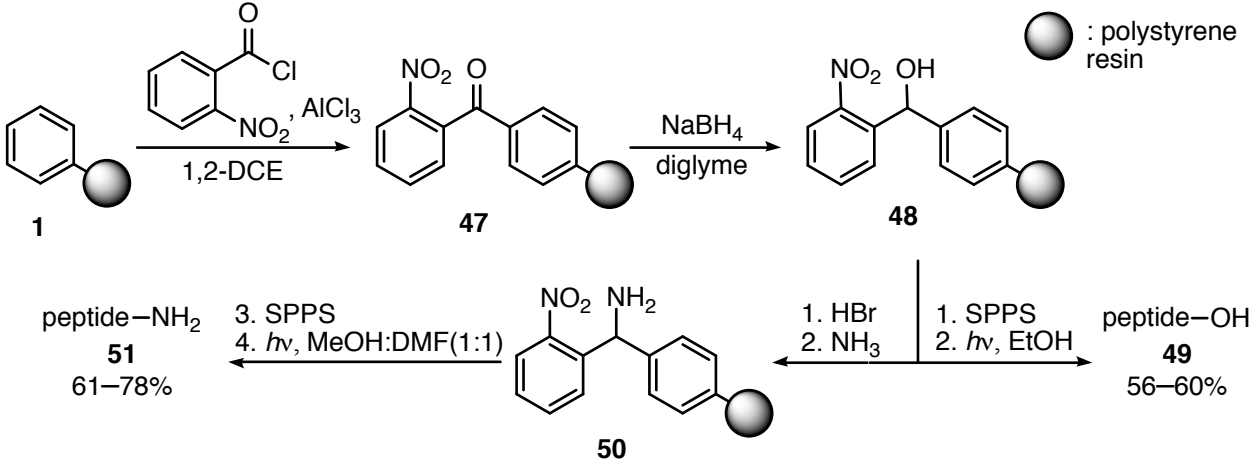


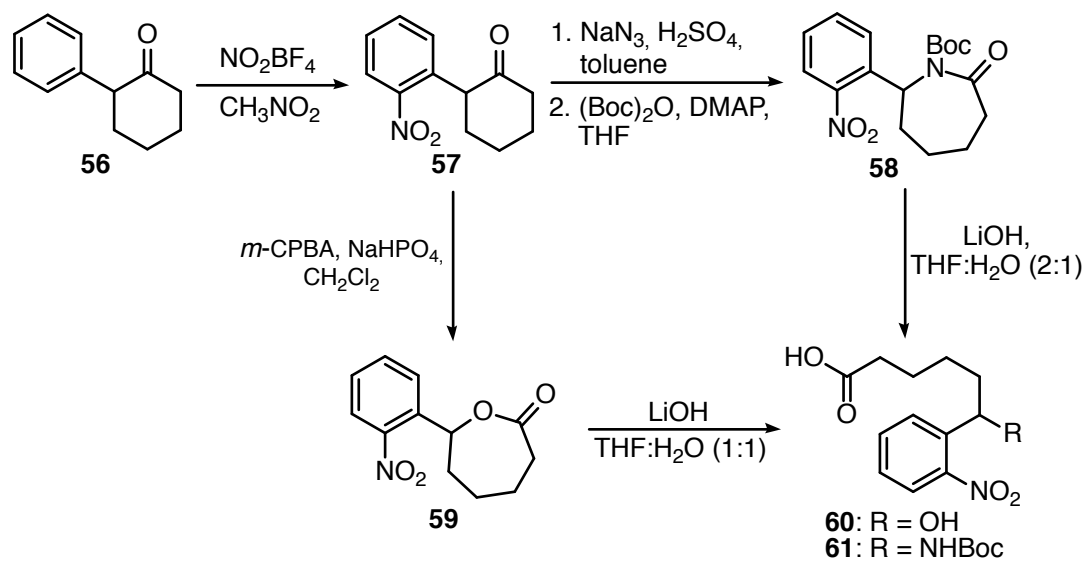




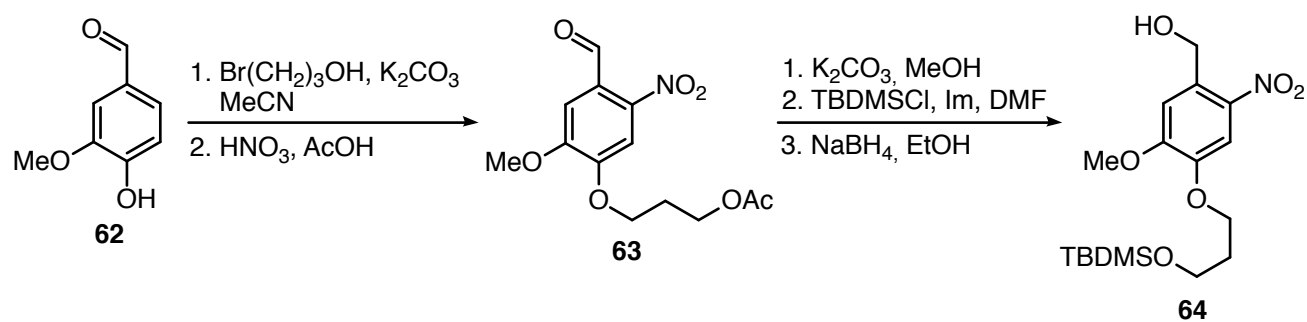


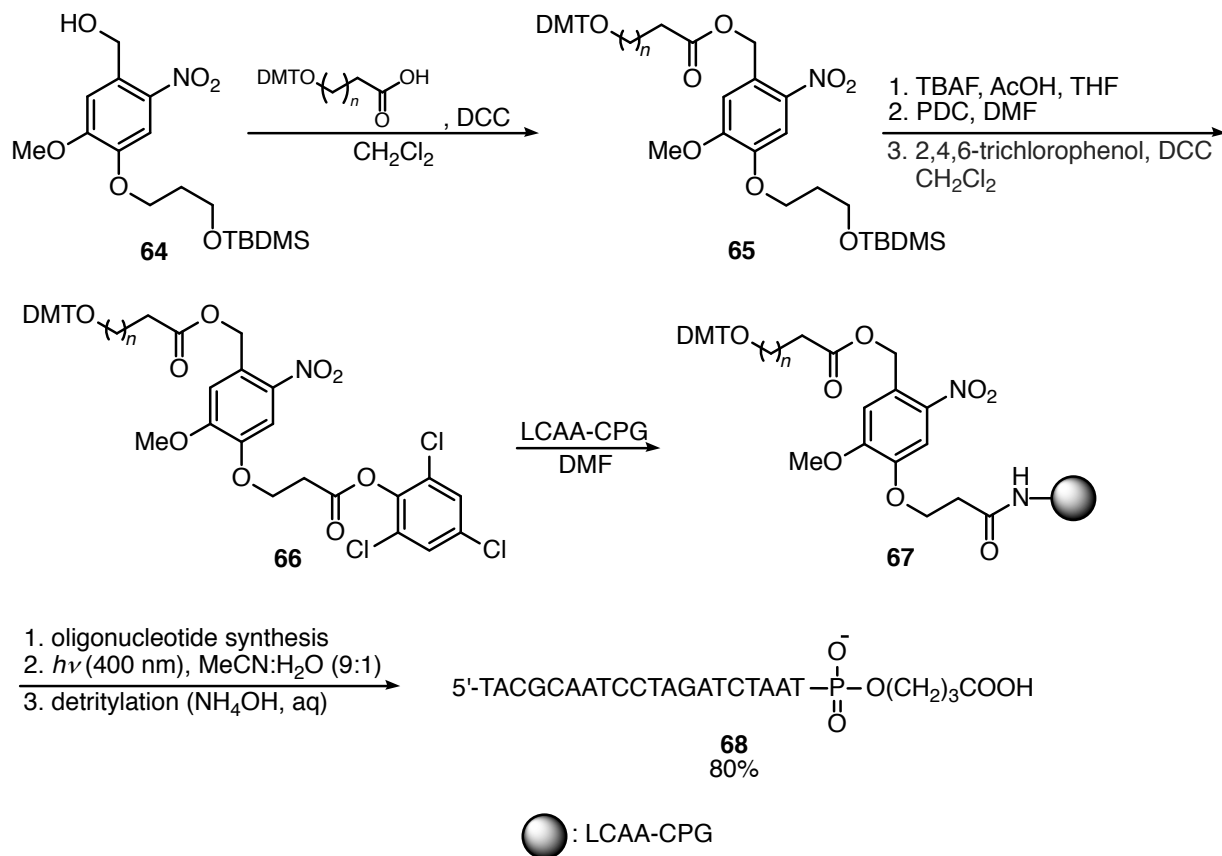


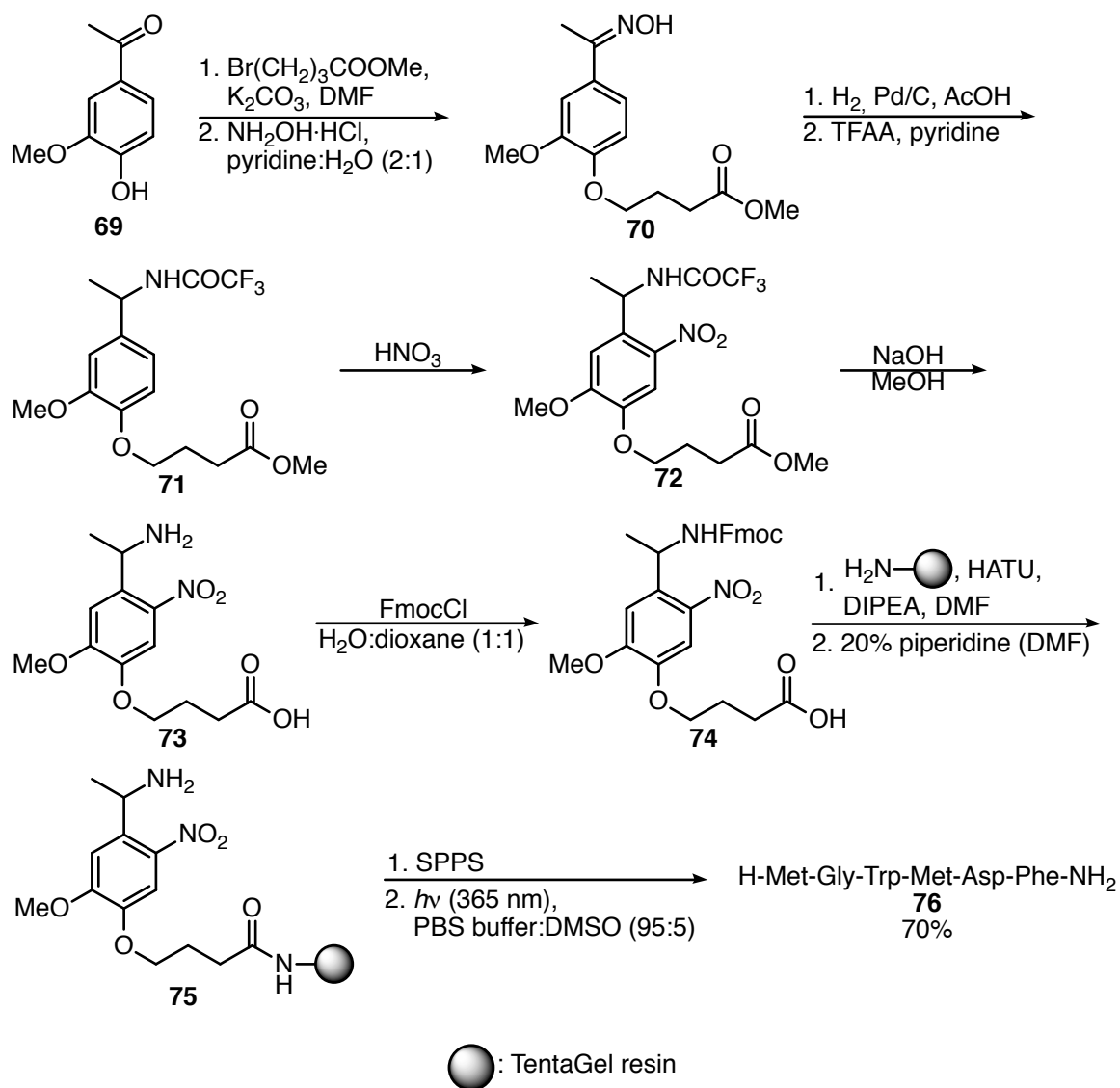


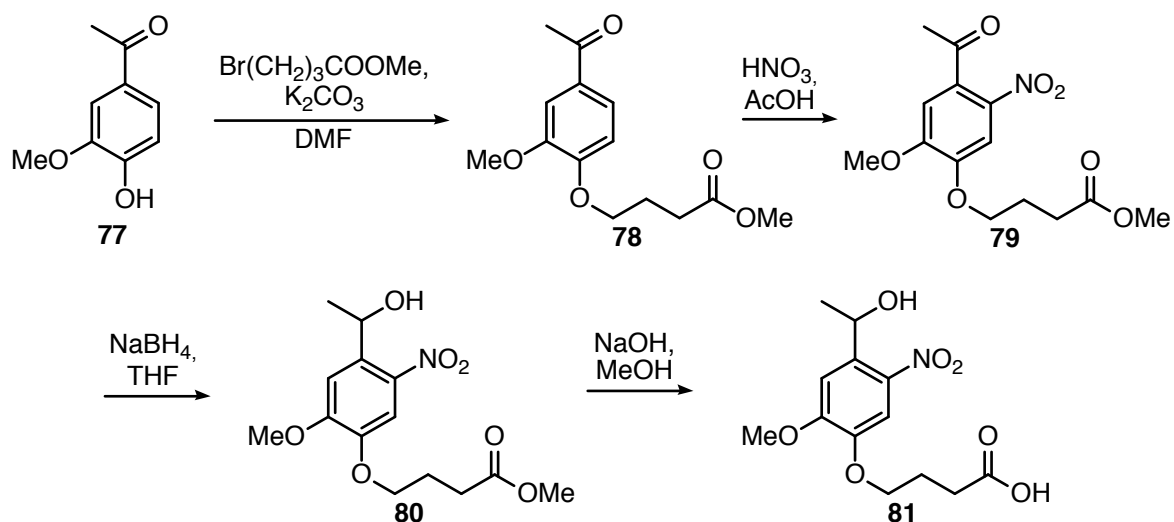


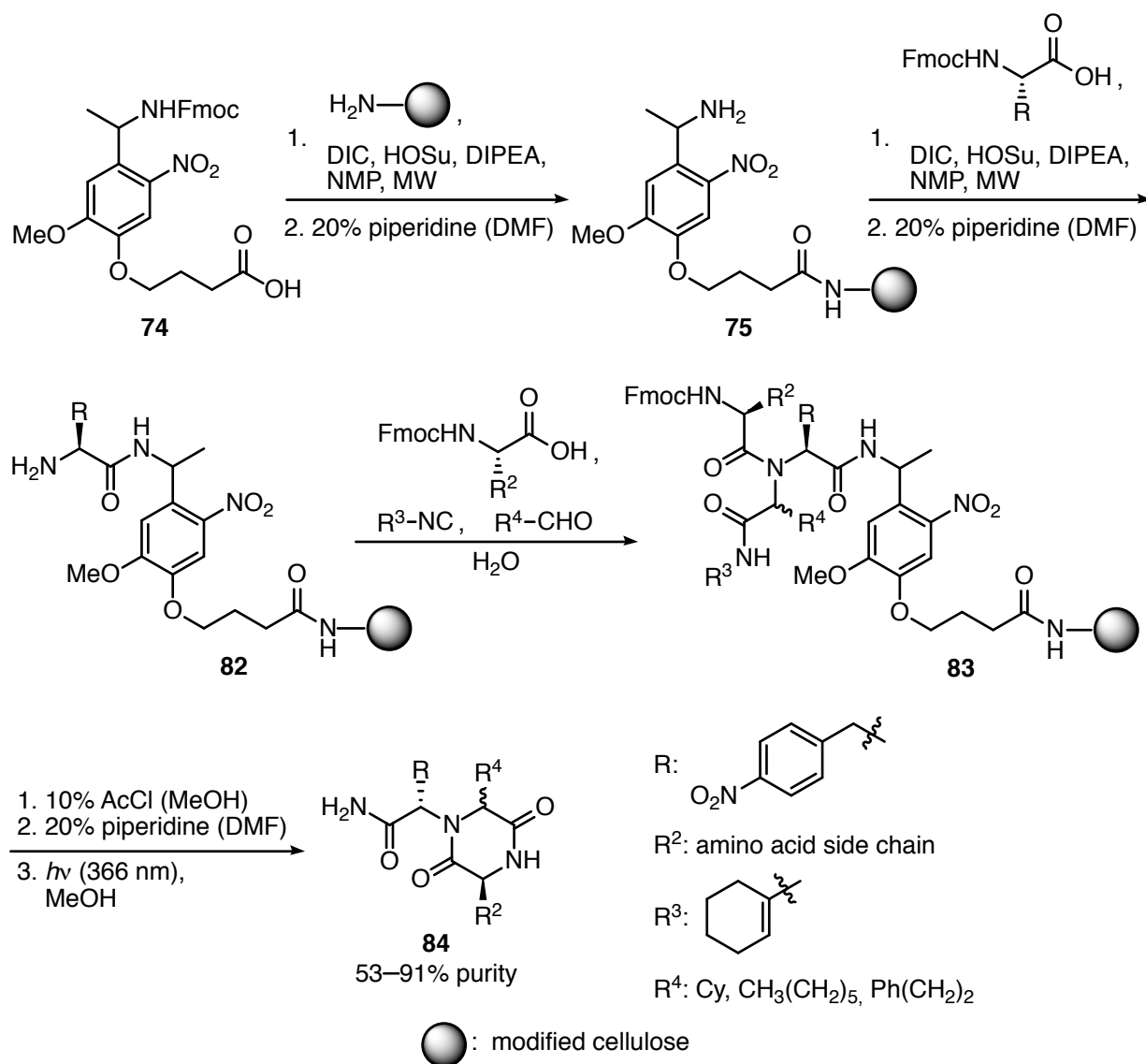
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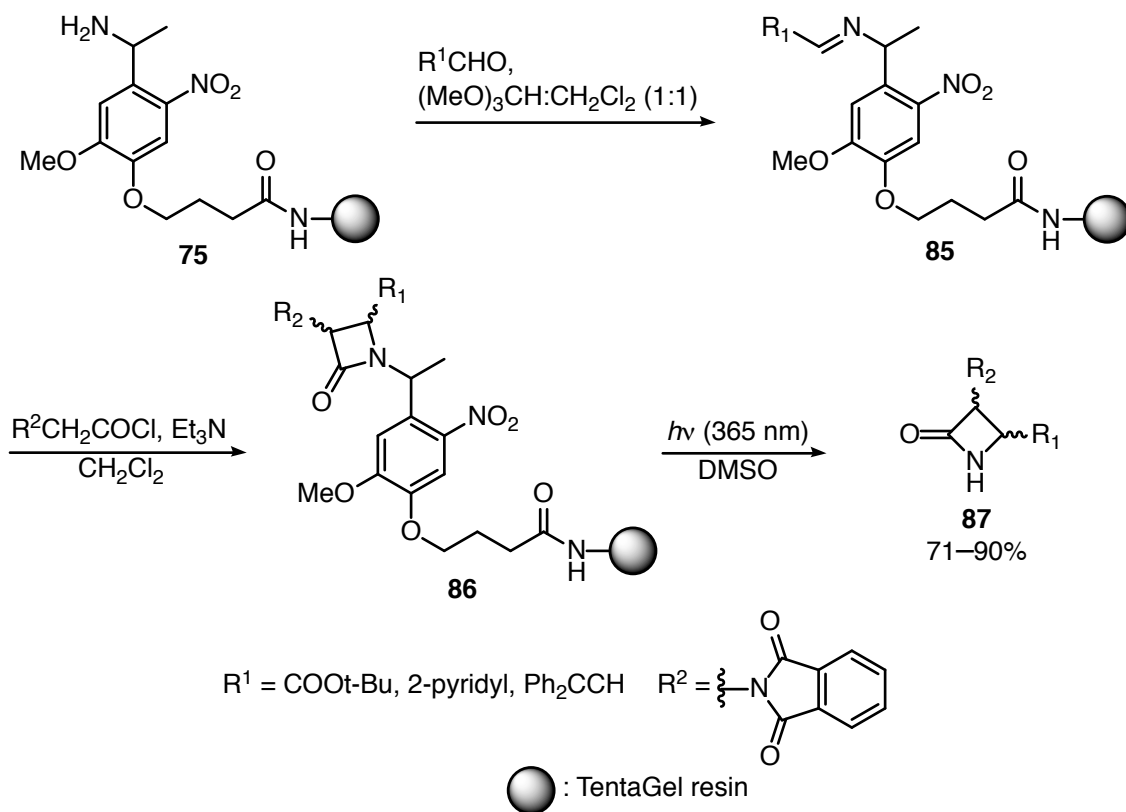


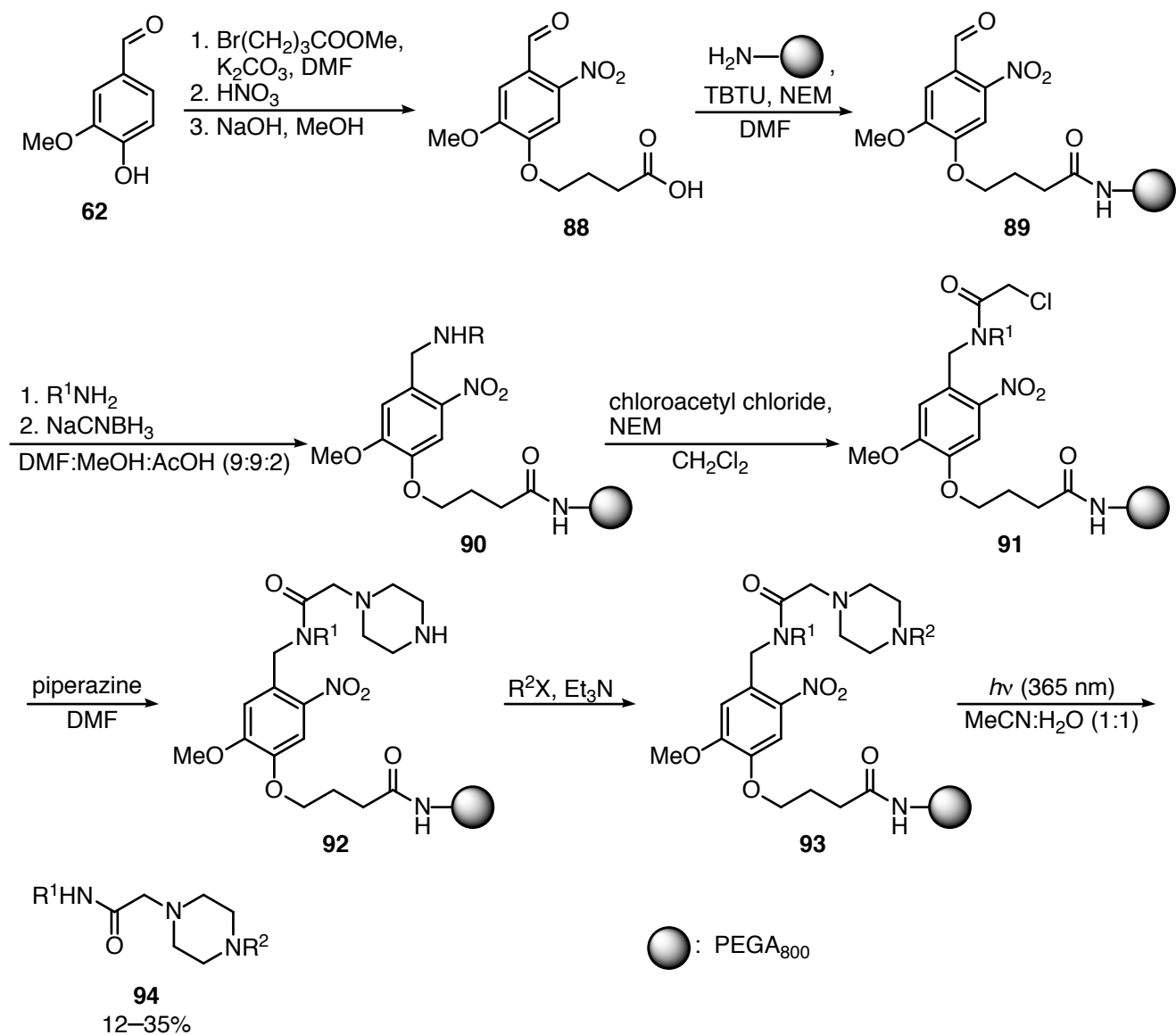


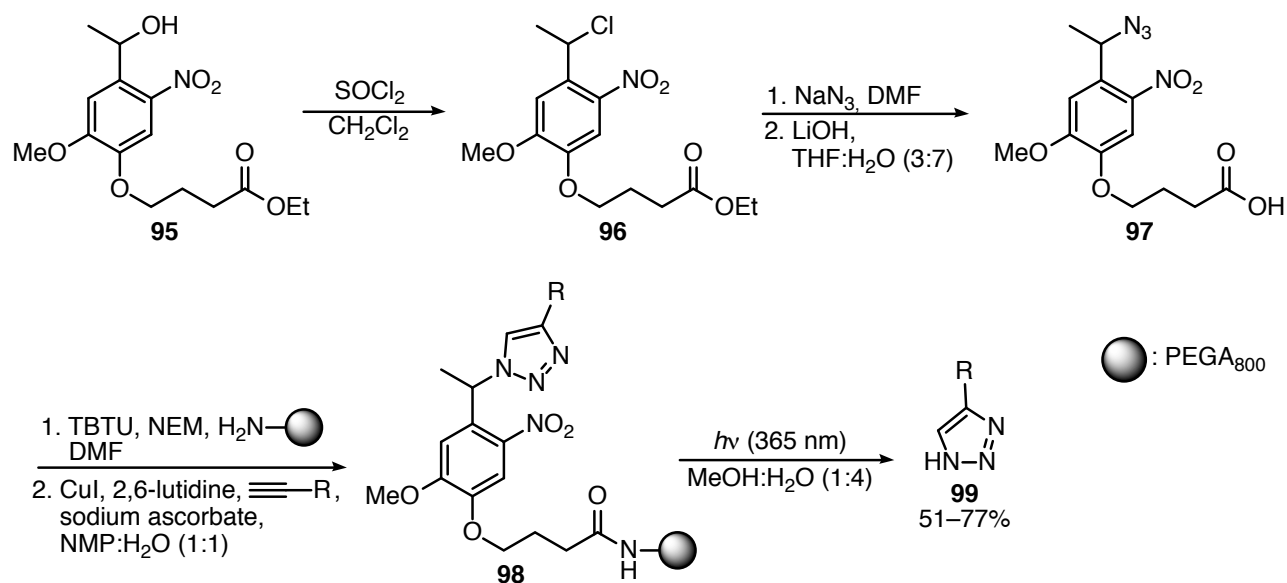




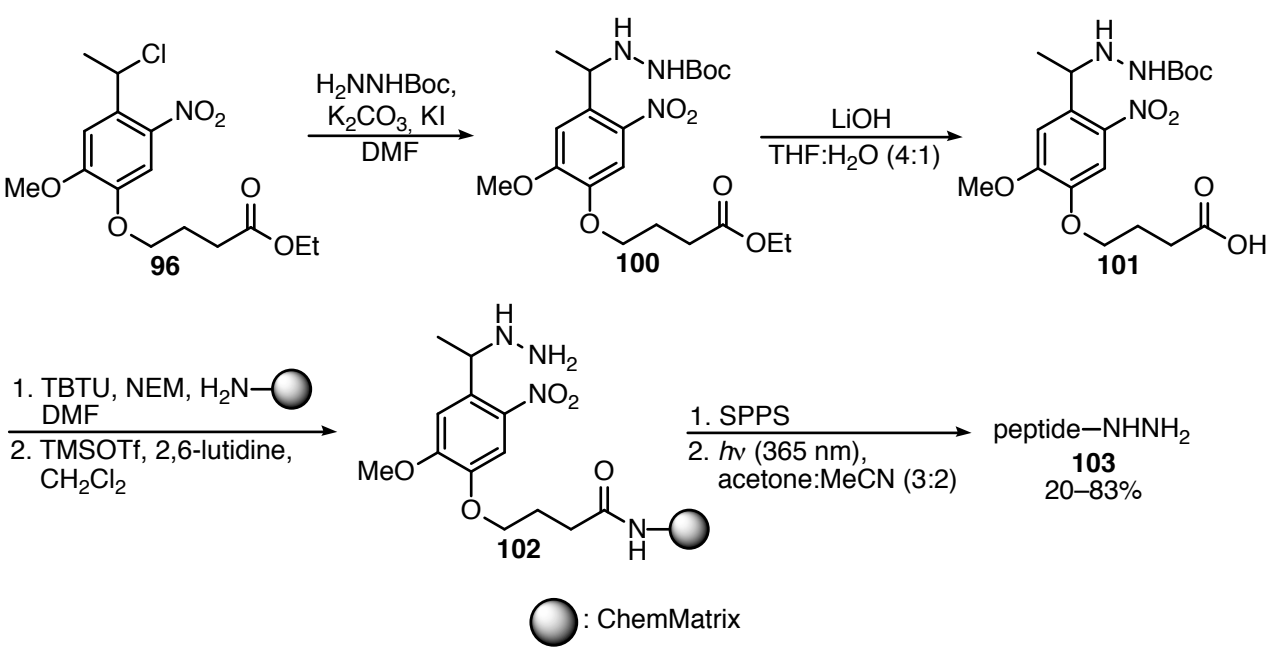


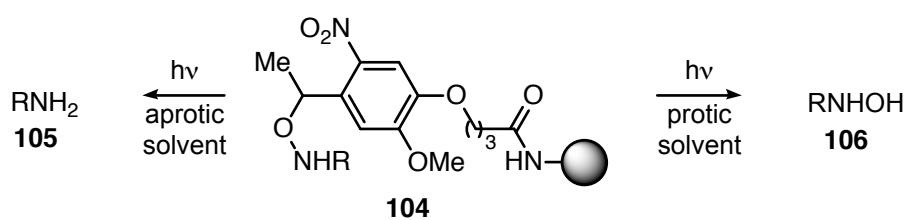


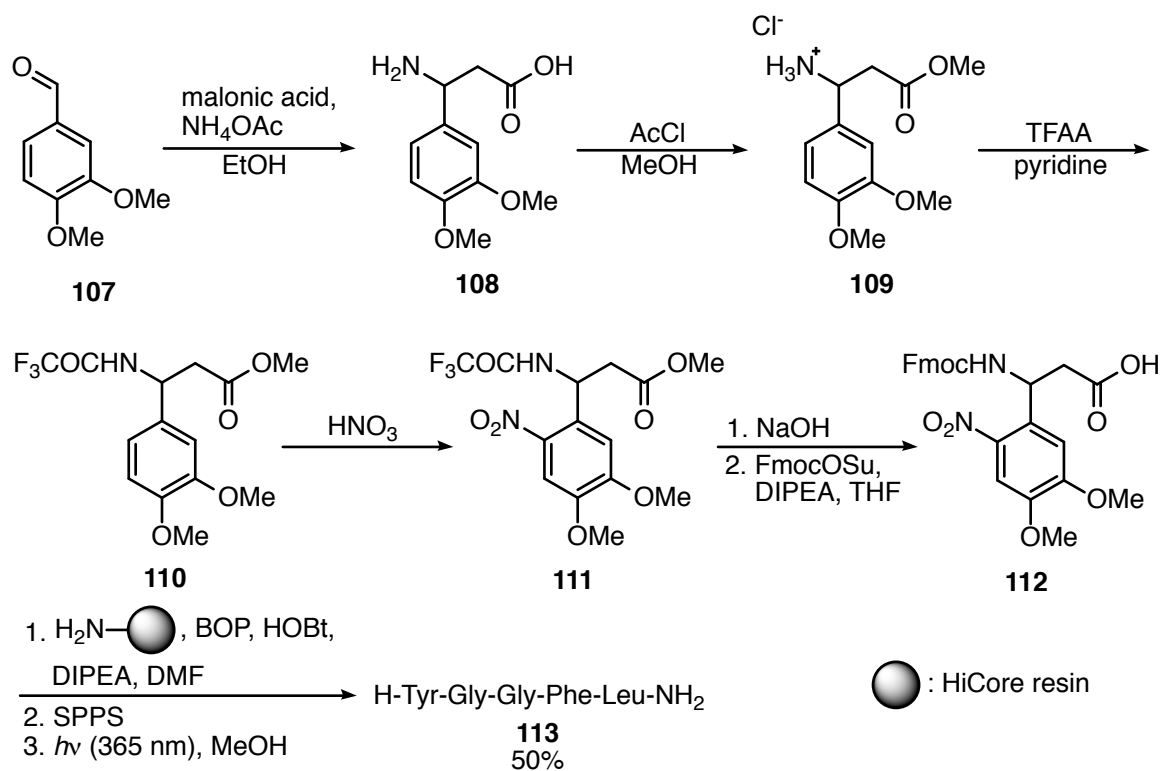


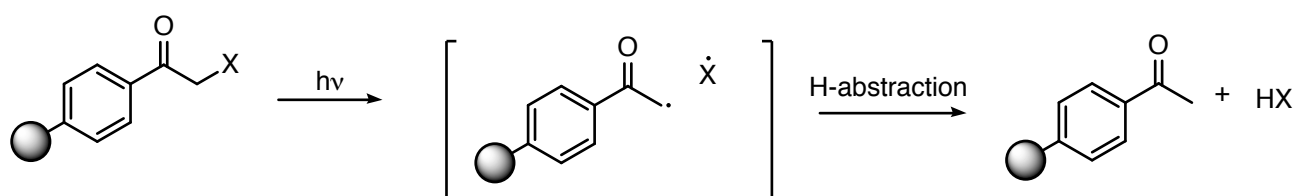


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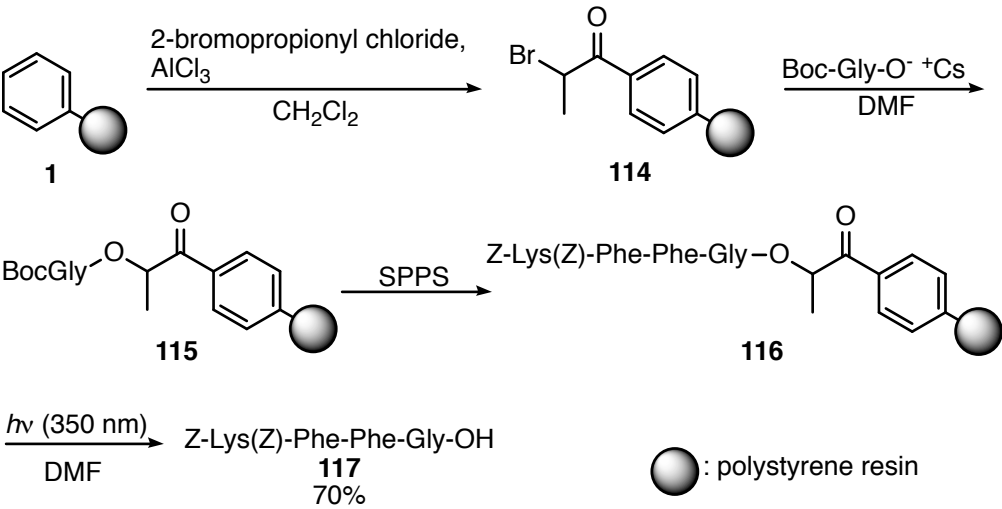


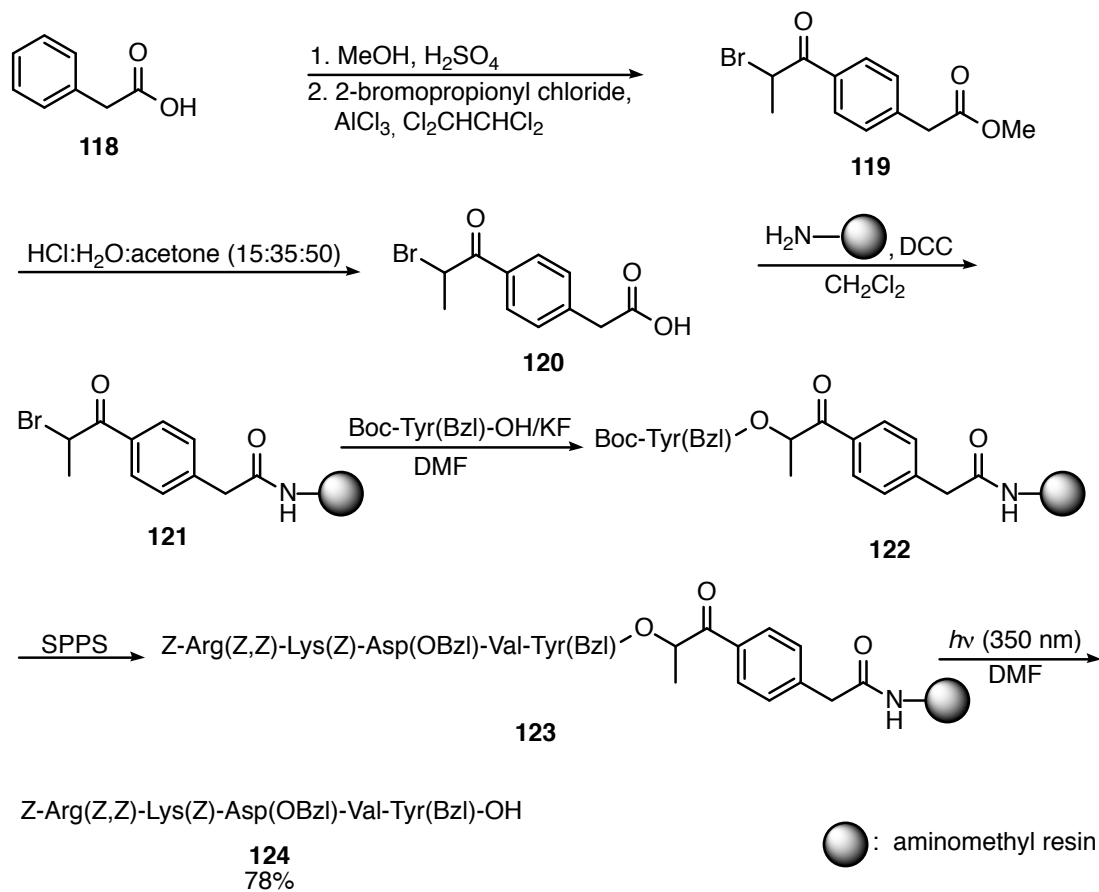


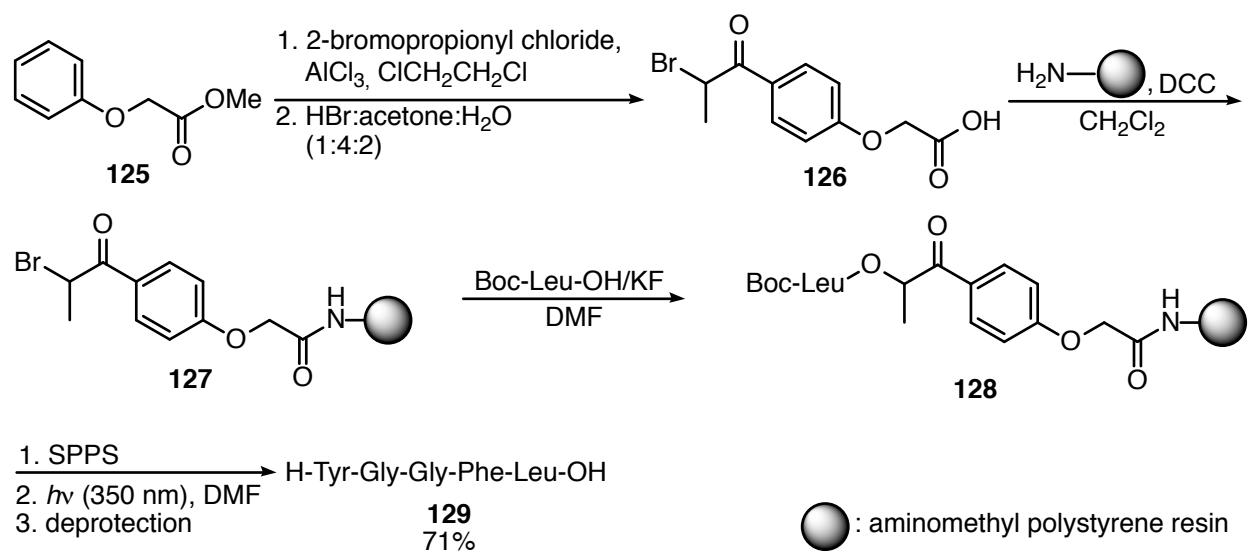




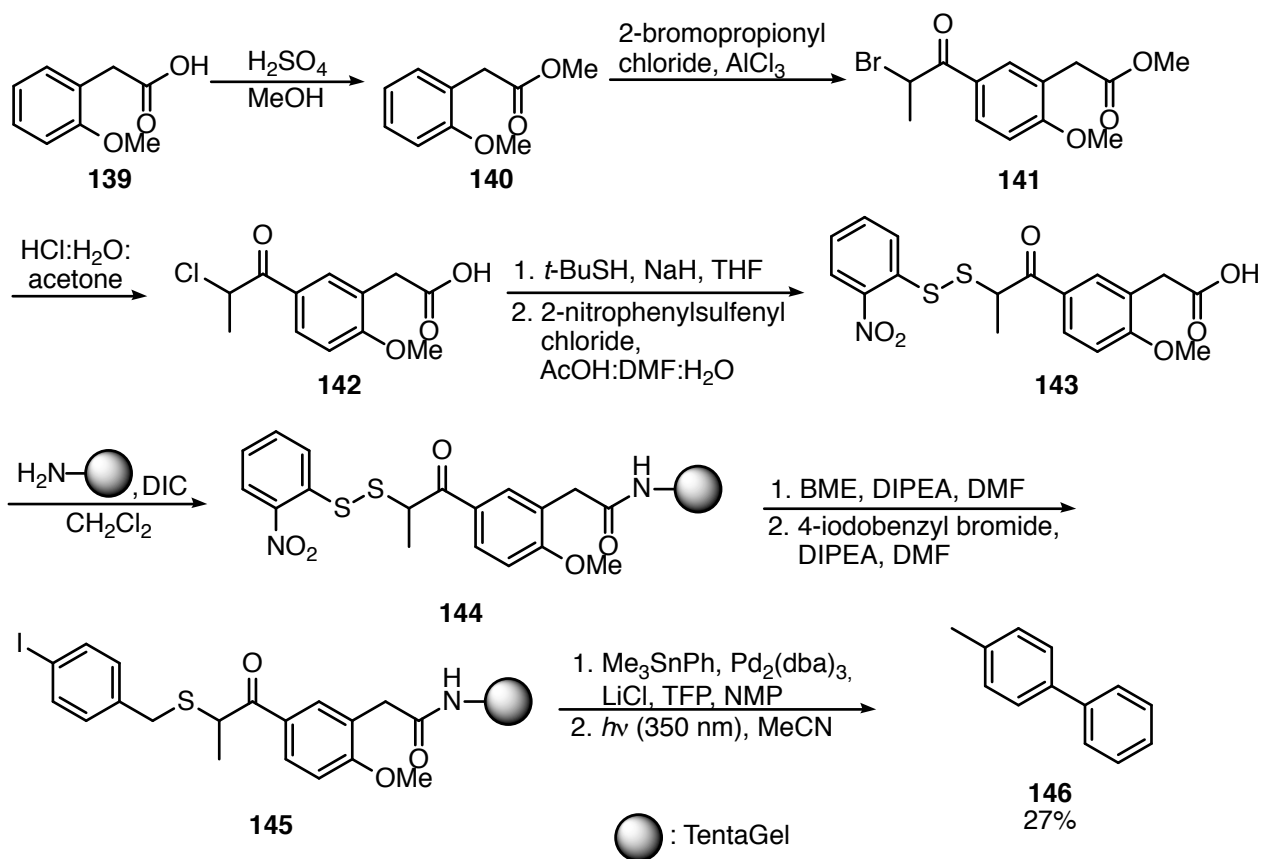
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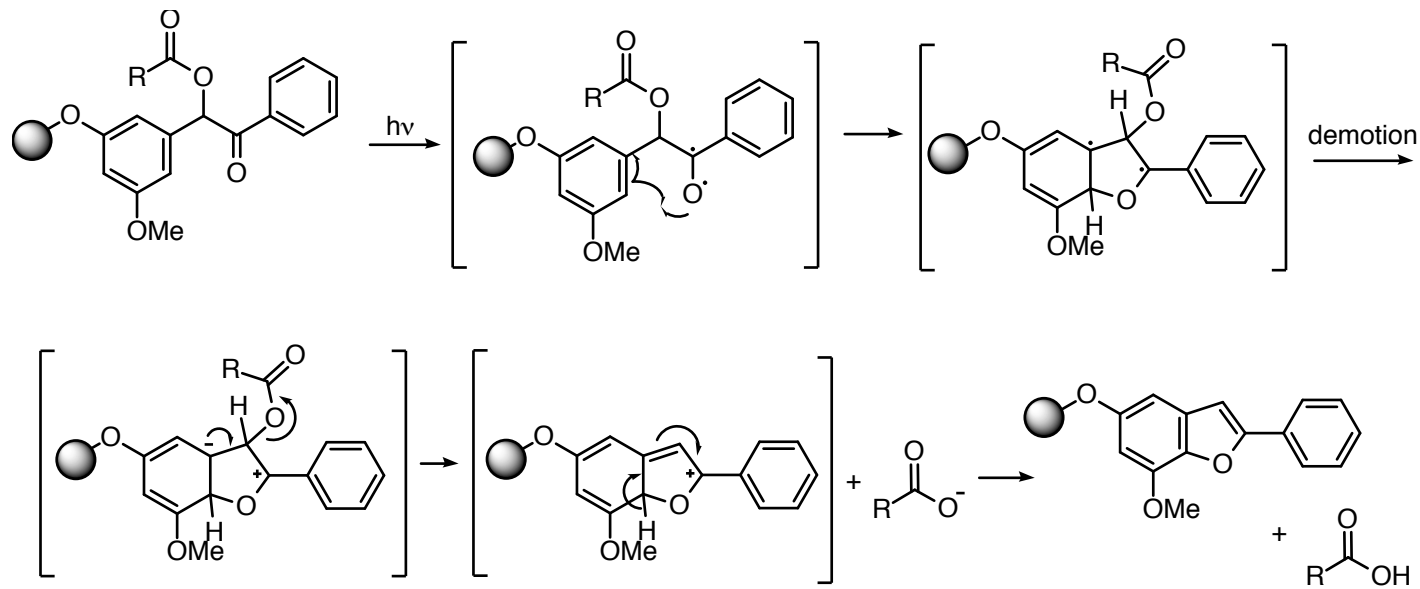


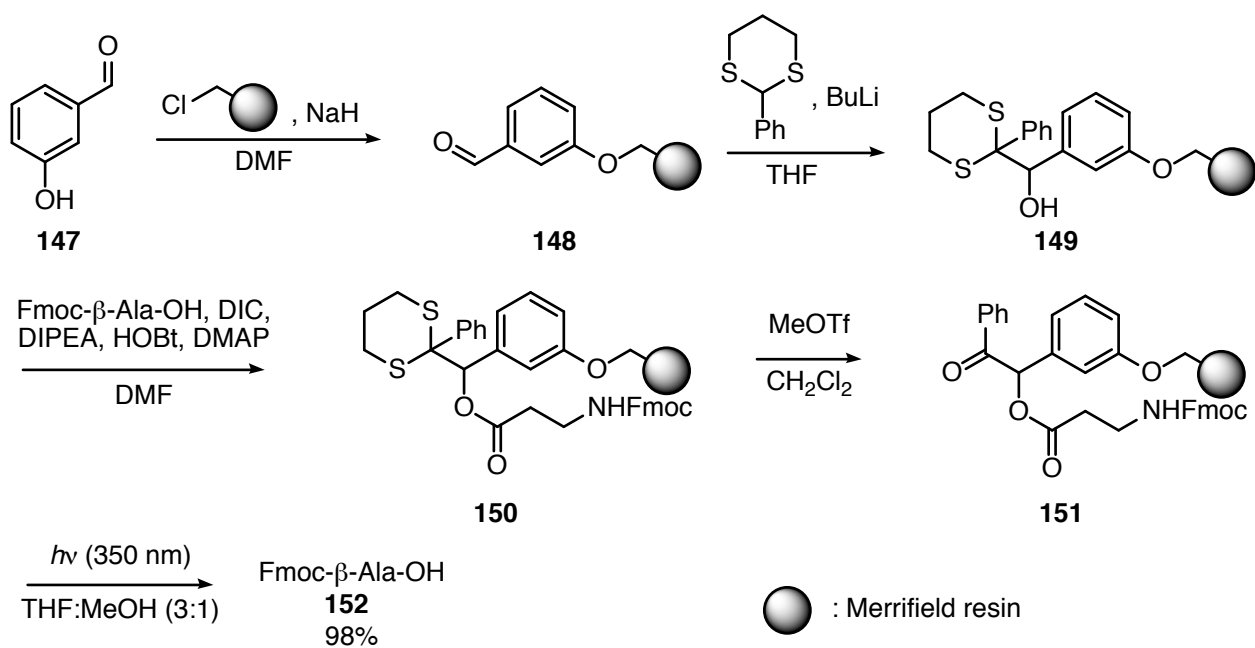


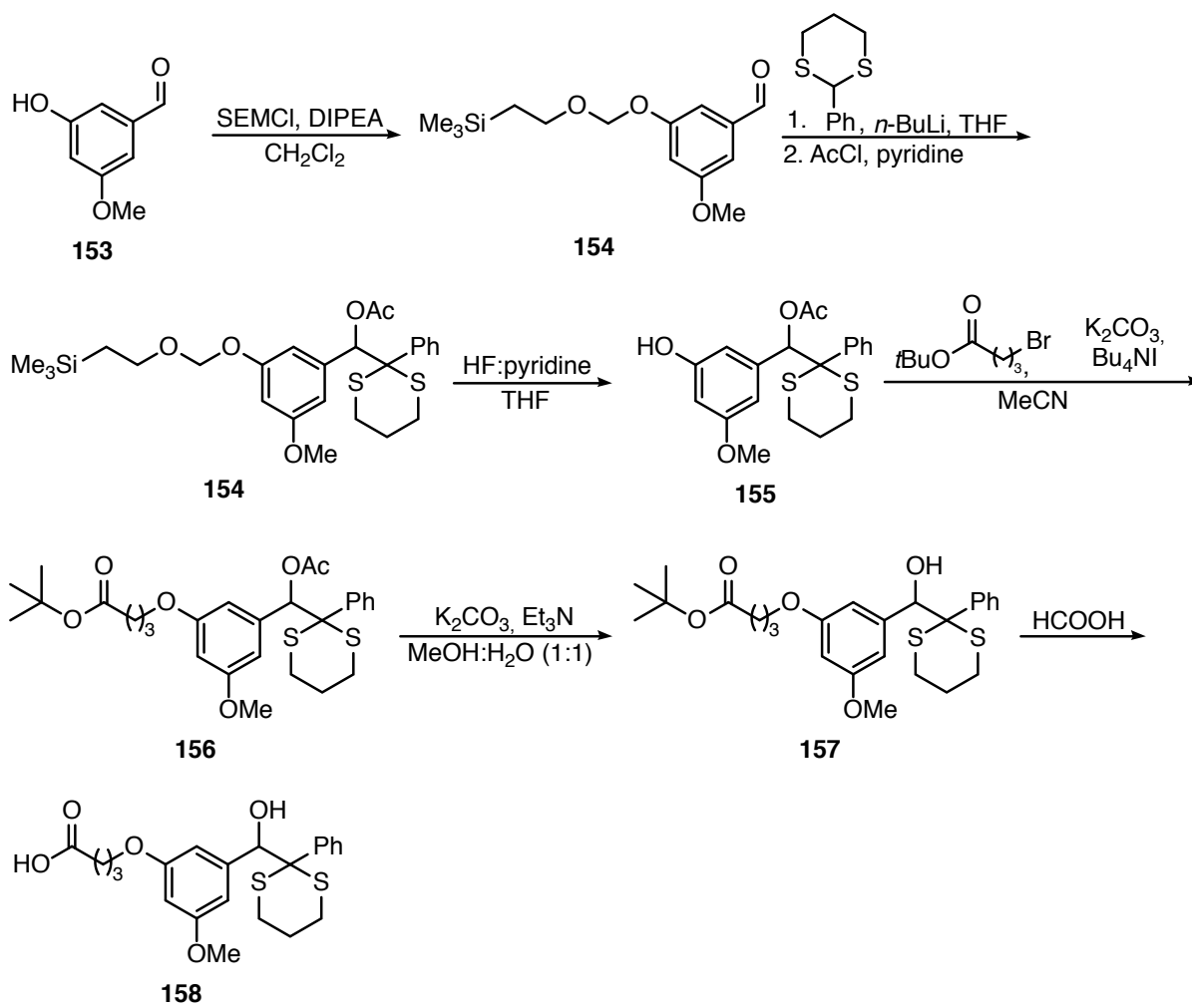


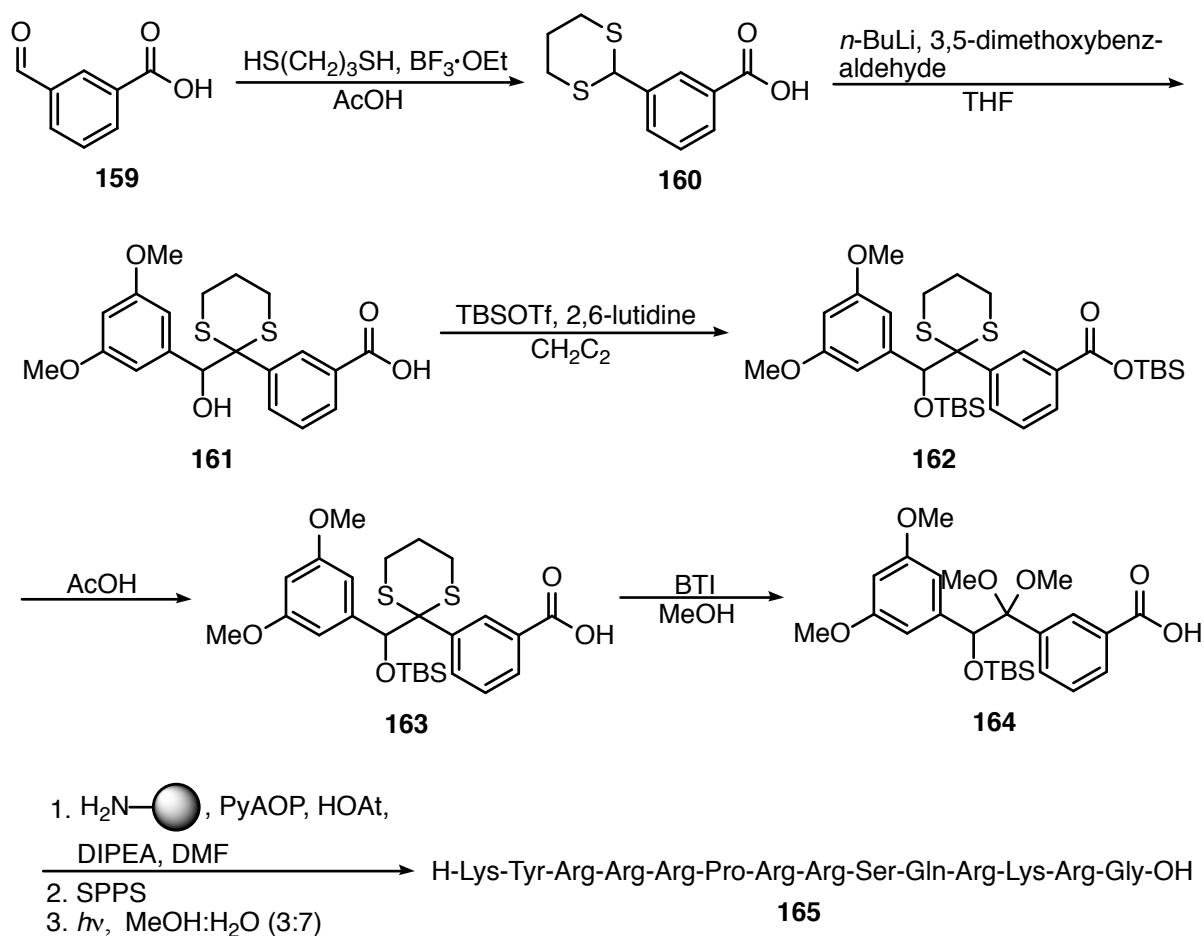






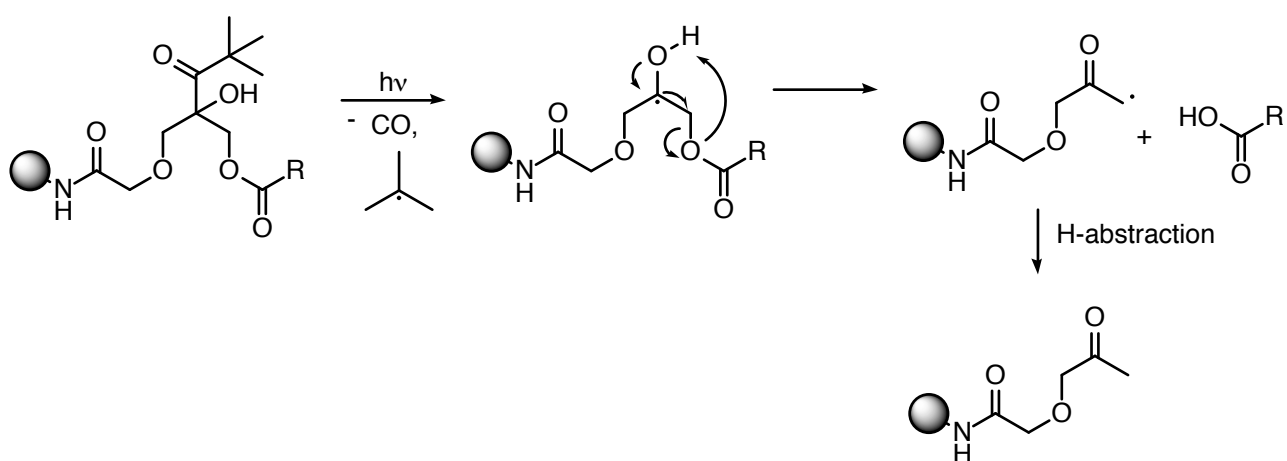


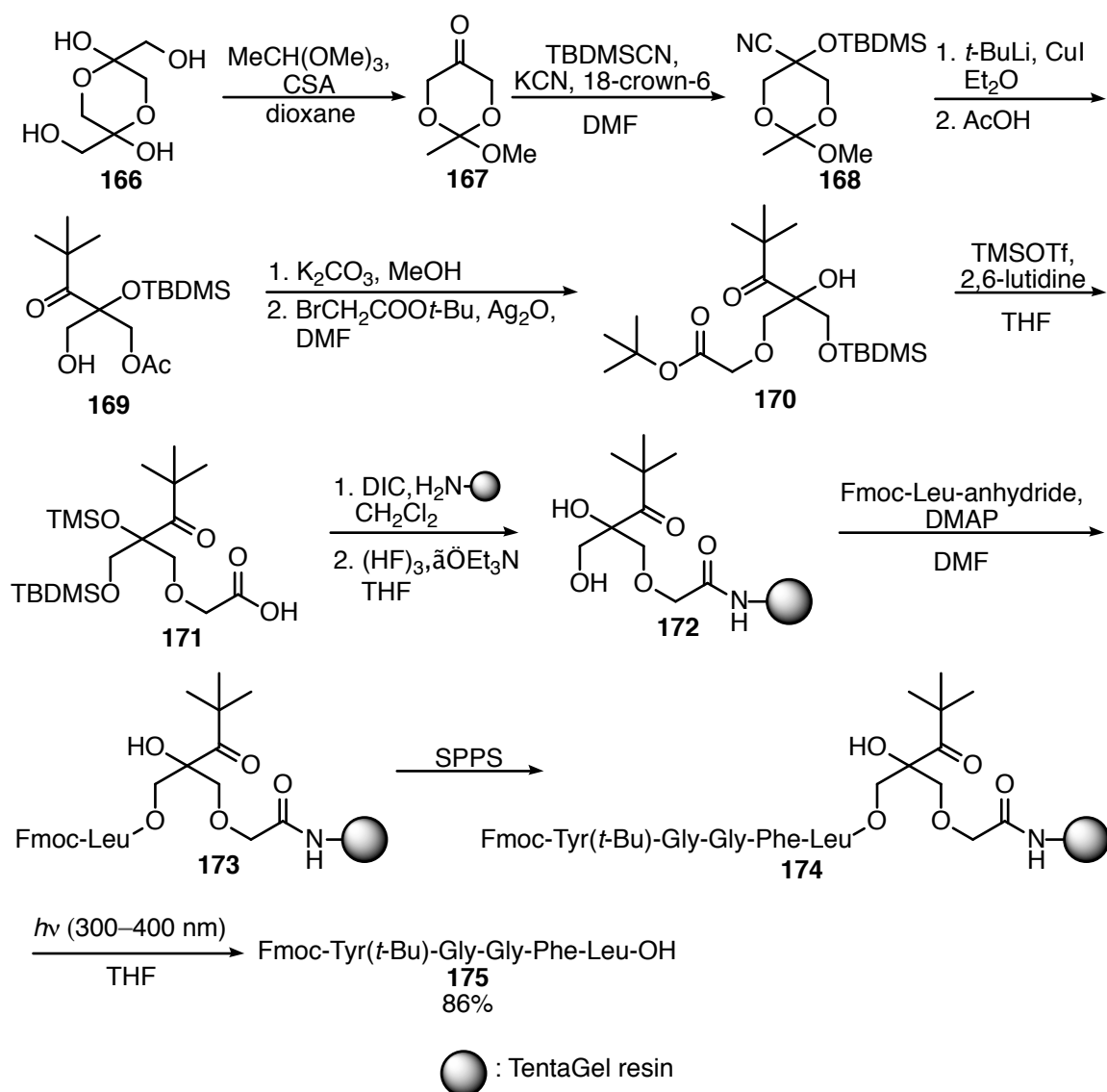


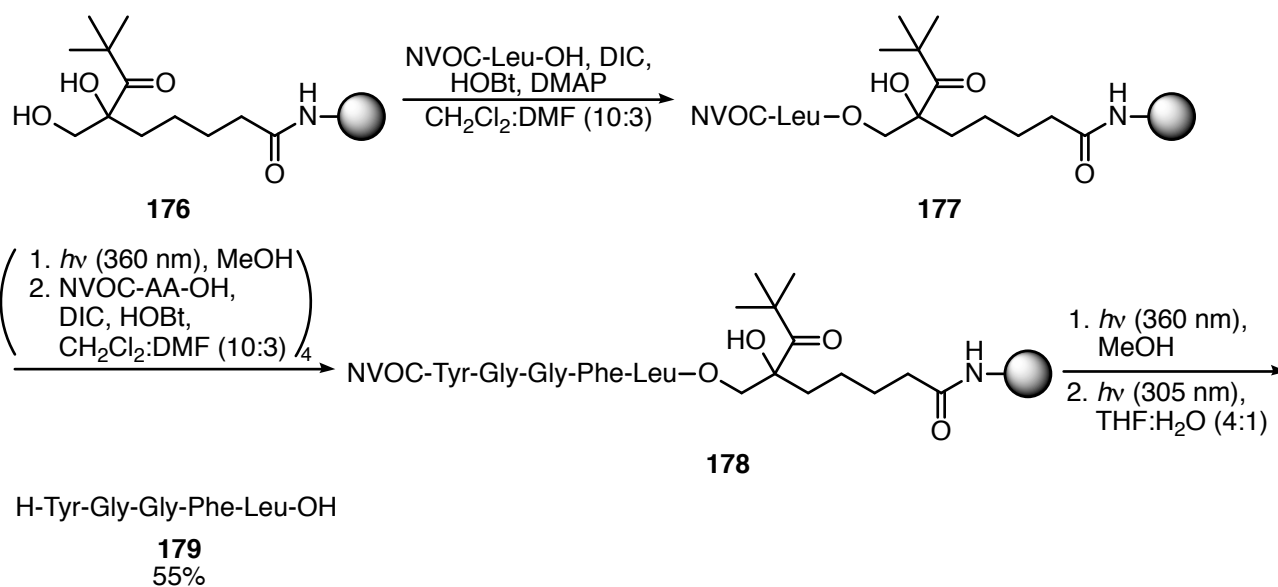



: TentaGel resin

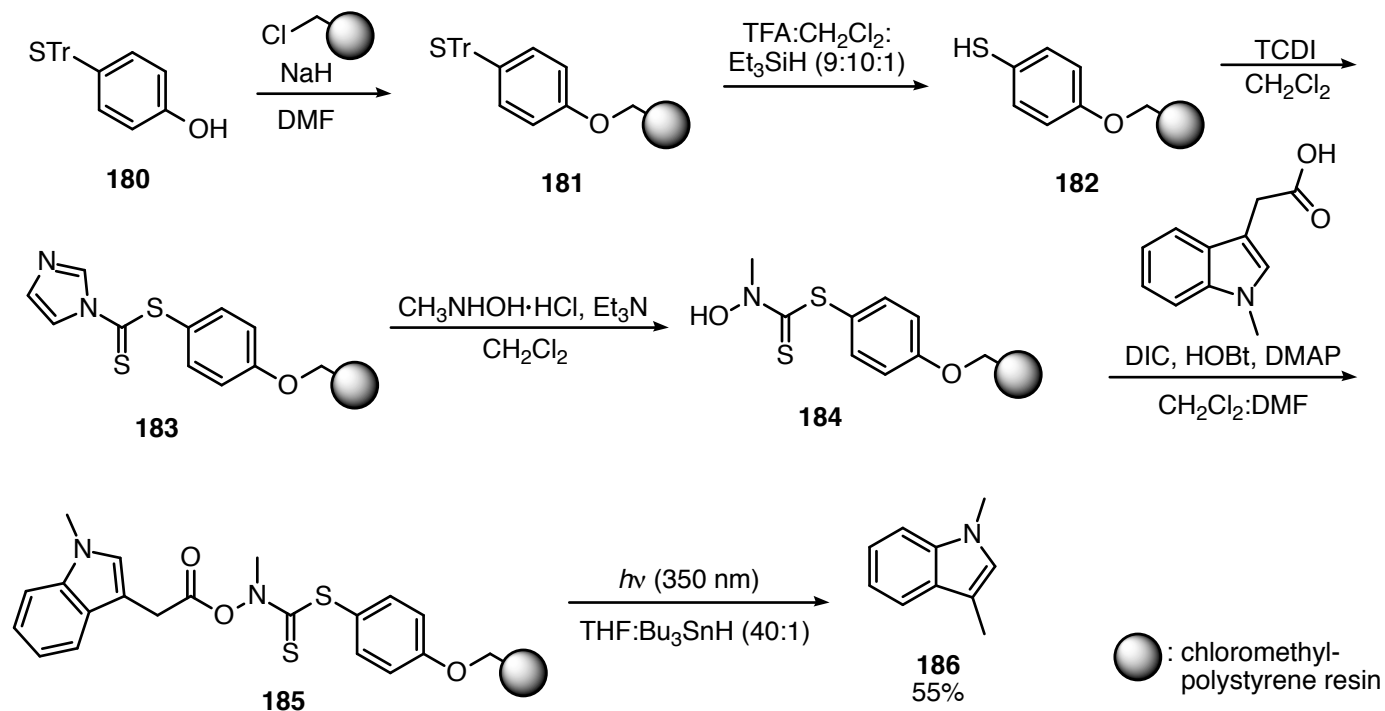
BTI: [bis(trifluoroacetoxy)iodo]benzene

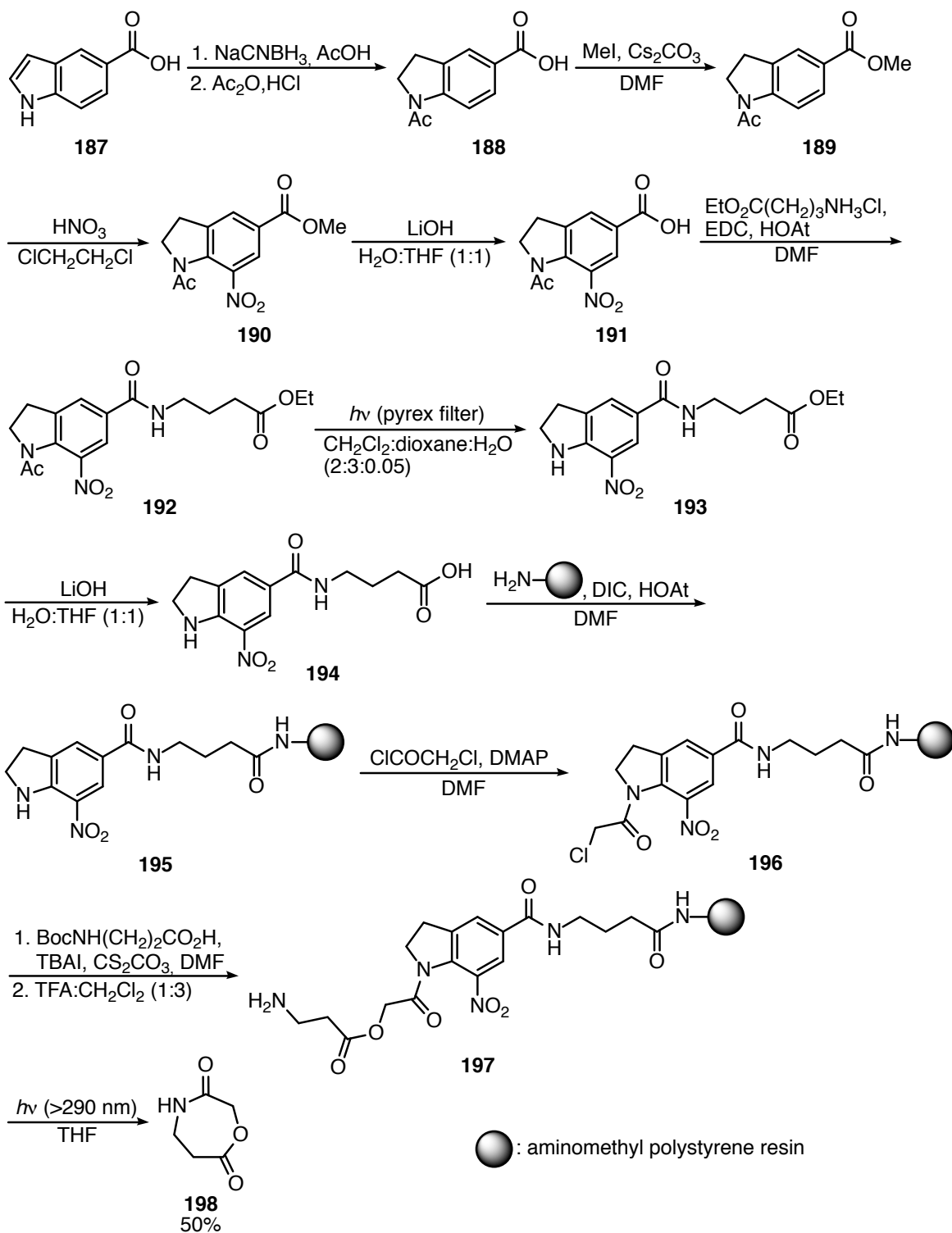


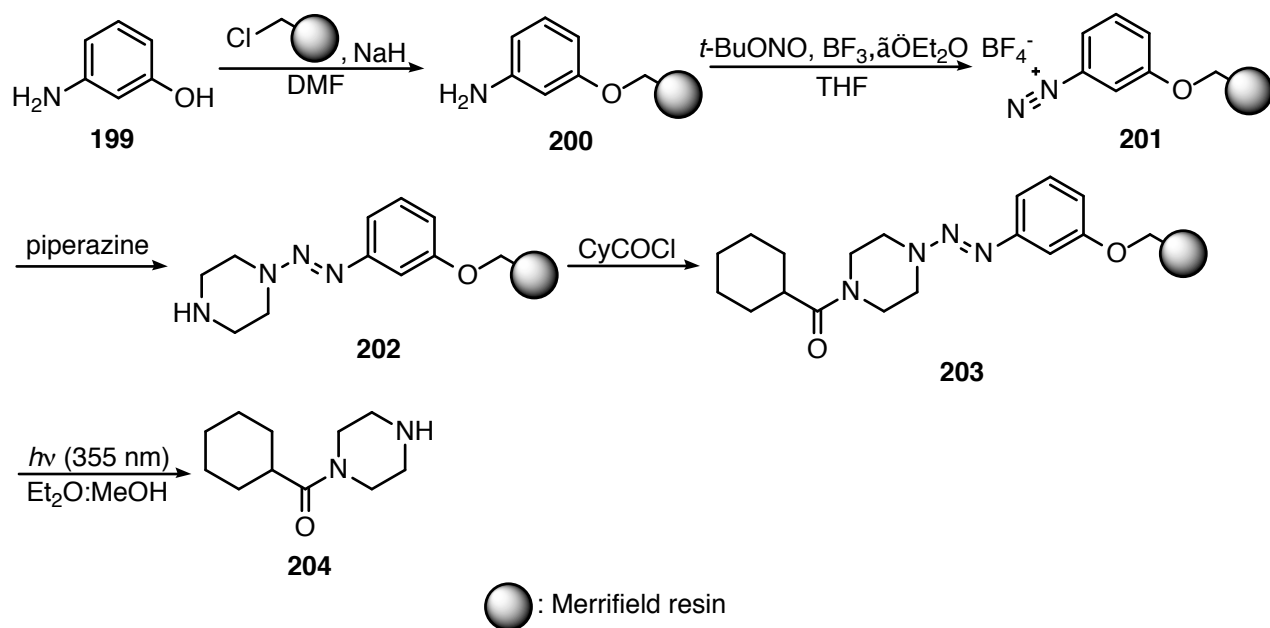




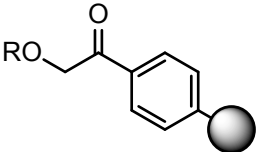
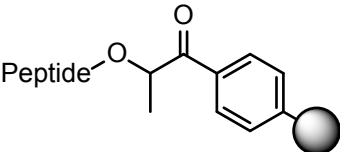
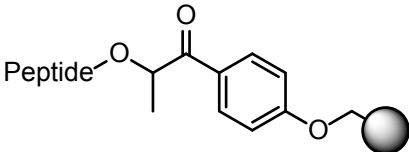
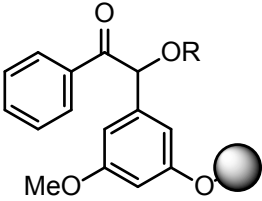
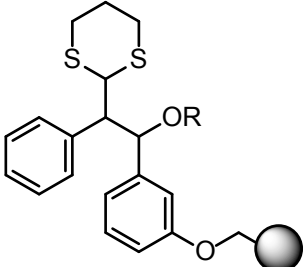
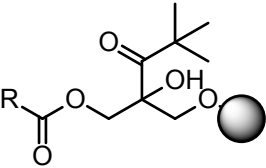
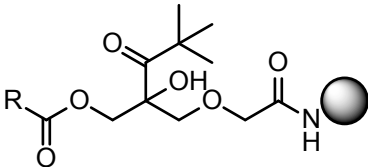
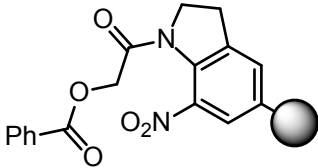
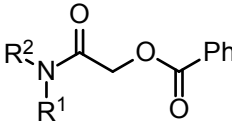
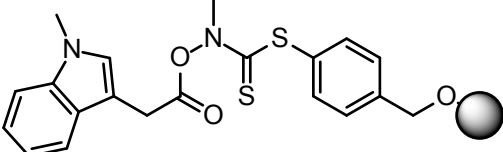
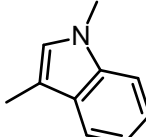
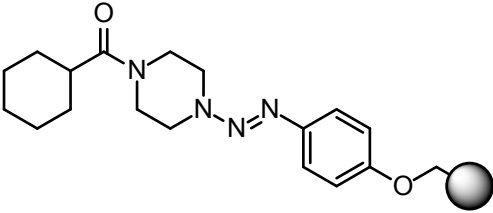
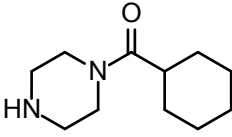
 : TentaGel resin







Linker	Cleavage Conditions	Product	References
<i>o</i>-Nitrobenzyl linkers			
	$h\nu, \lambda = 350 \text{ nm}$	HO-Gly-Tyr-Ser-Boc	[10]
	$h\nu, \lambda = 350 \text{ nm}$	X = O: Peptides X = O: Nucleotides X = N: Amido peptides X = O: Small molecules X = O: Saccharides	[11-13, 24],[35] [20-22] [32-34,36] [19, 37] [25-28]
<i>o</i>-nitrobenzyl linkers			
	$h\nu, \lambda = 350 \text{ nm}$	Amido peptides Glycosaminoglycans	[38-40]
	$h\nu, \lambda = 350 \text{ nm}$	Peptides	[41] [42]
<i>o</i>-Nitroveratryl linkers			
	$h\nu, \lambda = 350 \text{ nm}$	Nucleotides Saccharides	[49],[51-52] [48]
	$h\nu, \lambda = 365 \text{ nm}$	Amido peptides Sulfonamido peptides	[50] [59]
	$h\nu, \lambda = 365 \text{ nm}$		[60]
	$h\nu, \lambda = 365 \text{ nm}$		[62]
	$h\nu, \lambda = 365 \text{ nm}$		[63]
	$h\nu, \lambda = 365 \text{ nm}$		[64-65]

Linker	Cleavage Conditions	Product	References
Phenacyl linkers			
			
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	$h\nu, \lambda = 300\text{-}350\text{ nm}$	Peptides	[67-69]
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	$h\nu, \lambda = 350\text{ nm}$	Peptides	[70-71]
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Benzoin linkers			
			
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	$h\nu, \lambda = 350\text{ nm}$	Unnatural amino acids	[77]
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Pivaloyl linkers			
			
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	$h\nu, \lambda = 280\text{-}340\text{ nm}$	Peptides Small molecules	[80,82] [81]
<hr/>			
Other photolabile linkers			
	$h\nu, \lambda = 290\text{ nm}, R^1R^2NH$		[84]
	$h\nu, \lambda = 350\text{ nm},$ Bu_3SnH, THF		[83]
	$h\nu, \lambda = 355\text{ nm}$		[85]

Photolabile Linkers for Solid-Phase Synthesis

